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ÜBER DEN EINFLUSS VON BLEITRYPANBLAU AUF TEERKREBS BEI WEISSEN MÄUSEN. (Vorläufige Mitteilung).

Von *Svante Bursell*.

(Eingegangen bei der Redaktion am 26. Februar 1940).

Seitdem *Blaire Bell* seine ersten aufsehererregenden Arbeiten über Behandlung inoperabler Krebsfälle mit kolloidalen Bleipräparaten veröffentlicht hat, ist eine reiche Literatur auf diesen Gebiet erschienen. Wenngleich die vielen Nachuntersuchungen von mit verschiedenen Präparaten behandelten Fällen wechselnde Resultate ergeben haben, findet sich doch eine ganze Anzahl Arbeiten, in denen vielleicht nicht über eine ganz sichere Heilung, aber doch jedenfalls über eine merkliehe Besserung der Patienten bis zu voller Arbeitsfähigkeit berichtet wird. Wenn auch natürlich gleichzeitig alle mögliche anderen therapeutischen Mittel angewandt werden, um das Wachstum und die Verbreitung der Tumoren im Organismus zu bekämpfen, muss man doch den Bleipräparaten eine gewisse Bedeutung bei der sog. klinischen Heilung von inoperablen Kanzer zuschreiben. Indes ist noch keine Untersuchung veröffentlicht worden, in der an einem hinreichend grossen Material der Einfluss von Bleipräparaten allein auf Krebs unter Beurteilung des Resultats nach statistischen Methoden geprüft worden wäre. Die Vornahme einer derartigen Untersuchung dürfte daher von gewissem Interesse sein. Auf Anregung von Prof. med. Dr. *E. Louis Backman* habe ich deshalb eine Untersuchung über die Wirkung von Bleitrypanblau auf den durch

Teerpinselung bei weissen Mäusen hervorgerufenen Kanzer begonnen. Ich benutze die Gelegenheit, Herrn Prof. *Backman* meinen warmen Dank für sein wohlwollendes Interesse und seine wertvollen Ratschläge im Verlauf meiner Arbeit auszusprechen.*)

Blaire Bell nahm seine Untersuchungen an Patienten mit inoperablen Kanzer in verschiedenen Organen vor. Er war der Ansicht, dass Blei eine spezifische Wirkung auf die Kanzerzellen hat, weil geeignete Bleidosen Nekrosen im Chorionepitheliom hervorrufen. Ausserdem hatte er in ein paar Fällen mehr Blei im Tumor als im umgebenden Gewebe gefunden. Im Jahre 1929 veröffentlichte er eine Statistik über 566 behandelte Fälle; von diesen wurden 51 als geheilt angesehen, und in 12 Fällen war die Progression der Krankheit vollständig zum Stillstand gekommen. Zweifellos starben eine Anzahl Patienten an Bleivergiftung, wenigstens während einer Periode im Anfang der Untersuchung, als die Patienten ambulatorisch behandelt wurden. Deshalb ging man später zu Krankenhausbehandlung über, wobei das Blutbild und andere klinische Data sorgfältig verfolgt wurden.

An diese und früher veröffentlichte Arbeiten schlossen sich zahlreiche Nachuntersuchungen an Tieren und Menschen an. Man verwendete verschiedene organische Bleipräparate, eventuell in Verbindung mit gleichzeitiger Radium- oder Röntgenbehandlung. Viele Forscher konnten ermutigende Resultate verzeichnen, wenn auch nicht so gute wie *Bell*, während andere jede Behandlung mit Blei vollständig verwerfen, weil sie nicht nur unwirksam, sondern sogar schädlich sei.

Bargon, Horton & Osterberg benutzten kolloidales Bleiphosphat, eventuell in Verbindung mit Mangan oder Bleiselenid, welche letztere Substanz jedoch die Toxizität des Präparates erhöhte. Es wurden 83 Fälle von inoperablem Kanzer behandelt, von denen die meisten mehr als 400 mg Blei intra-

*) Die Untersuchungen sind mit Unterstützung von *Lotten Bohman's* Fonds für Krebsforschung, Stockholm, ausgeführt. Das verwandte Präparat ist von *Astra* in Södertälje hergestellt und gütig mir kostenlos gegeben, wofür ich meinen warmen Dank aussage.

venös bekamen. 14 Patienten waren zwei oder mehr Jahre später klinisch frei von Kanzer, 7 von diesen waren jedoch gleichzeitig mit Radium oder Röntgen behandelt worden. *Brunton* behandelte 36 Patienten mit einem aus Liverpool bezogenen organischen Bleipräparat S 7. Zwei der Patienten waren nach 3 Jahren klinisch gesund. *Fitzwilliams* behandelte 60 Fälle ausschliesslich mit Bleipräparat und erzielte ermutigende Resultate. *Stone Craver* behandelten 21 Patienten mit einem von *Woodward* hergestellten kolloidalen Bleipräparat; dabei bekamen sie in 8 Fällen regressive Veränderungen in den Tumoren, von denen zwei als temporär klinisch geheilt betrachtet wurden. Die Verfasser sind der Ansicht, dass eine Dosis von 1,5—2 mg Blei die Sicherheitsgrenze ist, welche eingehalten werden muss, um Vergiftungserscheinungen zu vermeiden. *Duroux* erzielte bei Verwendung von kolloidalem Blei mit gleichzeitiger Bestrahlungstherapie Heilung in zwei Fällen und ausserdem kürzere oder längere Besserungen, fast immer eine Besserung des Allgemeinzustandes sowie Verschwinden von Schmerzen und Blutungen. Inwieweit diese Erfolge dem Bleipräparat zu verdanken sind, ist unsicher, aber *Duroux* nimmt doch an, dass Bleibehandlung ein geeignetes Adjuvans der Strahlentherapie ist. *Knox* behandelte 60 Patienten mit festgestellter maligner Krankheit. Viele von ihnen erhielten gleichzeitig Röntgenbehandlung. Sie bediente sich hauptsächlich der 1926 von *Bell* angegebenen Methode. Nach zwei Jahren waren 5 Patienten am Leben, davon 3 in gutem Gesundheitszustand. Die Verfasserin bezeichnet es als wünschenswert, dass die Behandelten relativ jung, nicht anämisch noch kachektisch sind und keine zu ausgebreiteten Metastasen haben. Ferner hebt sie hervor, dass eine kombinierte Blei- und Röntgenbehandlung bessere Resultate ergibt als eine dieser Methoden allein. *Todd* injizierte 155 Patienten unter gleichzeitiger Strahlenbehandlung intravenös Bleiselenium in kolloidaler Form; ein Teil von diesen war jedoch moribund nach vorhergegangener erfolgloser Strahlenbehandlung. Von den übrigen schienen 24 nach zwei Jahren gesund zu sein. Sechs starben an interkurrenten Krankheiten, und bei diesen war bei der Sektion kein Tumor zu entdecken. *Ullman* gab drei Jahre lang Bleibehand-

lung als Adjuvans von Strahlenbehandlung. Verschiedene Fälle genesen und waren 6 Monate bis 2 Jahre nach Abschluss der Behandlung noch gesund. *Ullman* glaubt bemerkt zu haben, eine grössere Bleidosis stärker wirkt als mehrere kleine.

Eine Anzahl Autoren haben unsichere oder völlig negative Resultate erhalten. *Dalamier & Schwartz* verwendeten zwei verschiedene organische Präparate, von denen das eine dieselbe chemische Konstitution hatte wie das von *Bell* geprüfte. 17 Fälle wurden behandelt, aber nur 5 von ihnen in genügender Weise verfolgt. Die Verfasser konnten keine andere Wirkung beobachten als eine leichte Regression der Tumoren im Beginn der Behandlung. Der Krankheitsverlauf im Ganzen änderte sich nicht. *Polichetti* behandelte 38 Fälle von Kanzer in verschiedenen Organen mit kolloidalen Bleipräparaten, hergestellt von *Ganassini*, konnte aber keinerlei günstige Wirkung feststellen, nicht einmal auf das Allgemeinbefinden der Patienten. *Aub* behandelte 12 Fälle von Cancer mammae mit einem kolloidalen Bleiorthophosphat und gleichzeitiger tiefer Bestrahlung. 5 von ihnen starben an Bleivergiftung. *Mattina* behandelte ohne sicheren Erfolg 20 Fälle von inoperablem Kanzer mit kolloidalem Blei in Dosen von 0,25—0,8 g. *Kaemmerer* hatte bei Behandlung von 14 Fällen mit 52 %igem Bleisulphid in kolloidaler Lösung absolut negatives Resultat. *Reinhard & Buchwald*, *Dilling* sowie *Kawata* suchten vergeblich eine Tendenz des Bleies nachzuweisen, selektiv auf das Wachstum von Tumoren zu wirken. Sie konnten kein Blei im Tumorgewebe feststellen. Hinzuzufügen ist ferner, dass man auf der »International Conference on Cancer« in London 1928 zu dem Ergebnis gelangte, dass Blei keine spezifische Eigenschaft in bezug auf Kanzer habe, und dass seine Anwendung wegen seiner toxischen Wirkung ausserordentlich gefährlich ist.

Auch an Tieren mit experimentell erzeugtem Tumor, im allgemeinen Impftumoren, sind mit wechselnden Erfolg zahlreiche Versuche ausgeführt worden. Es war leichter, eine hemmende Wirkung auf Impftumoren zu erzielen als auf Krebs bei Menschen, weshalb die Resultate bei Impftumoren schwer

zu beurteilen sind. So hat *Collier* 15 verschiedene organische und anorganische Bleisalze mit gutem Erfolg bei Erlich's Mauskarzinom verwendet. Auch bei Tumoren entstanden nach intratestikulärer Injektion von Brown-Pearce's Kaninchenkarzinom konstatierte er hemmenden Einfluss einer von British Coolids Ltd. hergestellten Verbindung, welche 0,5 % Blei enthielt, den Maustumor 1/63 zum Verschwinden zu bringen. Da er gleichzeitig Radiumbehandlung gab, schreibt er das Resultat einer charakteristischen sekundären Radiation des Bleies zu. Anderer Forscher konnten keinen hemmenden Einfluss von Blei auf Tiertumoren feststellen. *v. Pastinszky & Ottenstein* fanden nach intramuskulärer Injektion von Bleipräparat kein Blei im Tumorgewebe und zogen hieraus den Schluss, dass Blei keine Tumoraffinität besitzt. *Krebs & Clemmensen* behandelten Tumoren vor der Transplantation gleichzeitig mit ihr oder nach dem Auswachsen des Tumors mit den Bleipräparaten R 232 und R 237 B und konnten dabei keinen hemmenden Einfluss auf den Tumor beobachten. *Eggers* versuchte, das Tumorwachstum mittelst selektiver Vergiftung der Tumorzellen durch Zuckerverbindungen mit toxischen Radikalen zu hemmen. Flexner-Jobling's Rattenkarzinom, welches ziemlich gutartig ist, verschwand allerdings, aber das bösartigere Sarkom 39 blieb praktisch unbeeinflusst.

Ferner hat man den Effekt verschiedener tumoraffiner Farbstoffe auf Tumoren untersucht. *Forssman* hat eine ganze Reihe tumoraffinen Farbstoffen in Bezug auf ihre Einwirkung auf Teerkanzer bei weissen Mäusen untersucht. Diese Untersuchung eines grossen Materials gab jedoch keine ermutigende Resultate. Von allen geprüften Farbstoffen u. a. Isaminblau und Trypanrot zeigte sich nur Nachtgrün eine spezifische Tumoraffinität zu haben bei Verwendung der anderen blieben die Tumoren farblos. Hinsichtlich ihres Einflusses auf die Entstehung von Karzinomen haben sich alle untersuchte Farbstoffen als bedeutungslos erwiesen. Nach *Nyka* wirken Isaminblau und Pyrrolblau hemmend auf das Wachstum von Impftumoren. *v. Rosen* gibt an, mit Isaminblau günstigen Erfolg bei Menschen erzielt zu haben, teilt jedoch keine Kasuistik mit.

Kimura konnte keine Wirkung von Isaminblau oder Trypanblau auf Rattensarkom feststellen, während Isamincholin und Trypancholin in 21 Fällen von 60 Heilung herbeiführten. Von 30 Kaninchensarkomen heilten 13 vollständig aus. *Lewis & Lewis* prüften den Einfluss von 80 verschiedenen Farbstoffen auf den Kückentumor 1 und infektiöses Myxom bei Kaninchen, ohne ein sicheres Resultat konstatieren zu können. *Minegishi* konnte keinen Einfluss von Isaminblau auf Rattenkarzinom beobachten. *Russ & Scott* steigerten die Empfindlichkeit für Strahlenbehandlung durch vorbereitende Behandlung mit Trypanblau. Thorotrast hatte gleichartige Wirkung.

Man hat auch eine Reihe Versuche mit Kombinationen von tumoraffinen Farbstoffen und Metallen gemacht. Dabei ging man von dem Gedanken hervor, dass der tumoraffine Farbstoffe das Metall auf den Tumor übertragen und dadurch einen stärkeren Effekt des konzentrierten Metalls auf das Tumorstadium herbeiführen würde. *Zadik* gibt an, eine sehr gute Wirkung mit Wismut-Isaminblau erzielt zu haben. Er behandelte 109 Patienten mit insgesamt über 2000 Injektionen; dabei waren die Resultate bei Lungen-, Urogenital- und Ovarialkanzer ermutigend, während bei Ventrikel- und Darmkanzer keine Erfolge zu bemerken waren. *Woodhouse* prüfte eine Serie organischer Bleiverbindungen, u. a. Bleitrypanblau und Bleitrypanrot. Die Untersuchung erfolgte an durch Teerpinselung hervorgerufenen Tumoren weisser Mäuse. Die Herstellung der Farbstoffe wird eingehend beschrieben. Sie werden durch Gelatine in Lösung gehalten und subkutan oder intravenös injiziert. Ein hemmender Einfluss auf die Tumoren war nicht festzustellen. Dagegen starben die Versuchstiere viel früher als die Kontrolltiere, was darauf beruhen dürfte, dass sie so grosse Bleidosen bekamen.

Ich habe Bleitrypanblau nach *Woodhouse's* eigener Beschreibung hergestellt. Dieses Präparat enthielt 196,7 mg % Bleisulphat, entsprechend 134,4 mg % metallischen Blei, wobei das Blei teilweise an Gelatine adsorbiert war. Da die Dosierung bei intravenöser Behandlung 0,1 cc wöchentlich betrug, entsprechend 6,7 mg je kg Körpergewicht, bekamen die

Tieren bloss durch zweiwöchige Behandlung eine Gesamtdosis, welche *Bell's* höchsten Behandlungsdosen entspricht. Daher ist es nicht zu verwundern, dass sie durchschnittlich nicht länger als 12 Wochen lebten. Bei subkutaner Behandlung betrug die Dosis 0,2 ml einmal wöchentlich.

Ich bin von demselben Gedankengang ausgegangen wie die zuletzt erwähnten Arbeiten. Jedoch hatte *Woodhouse* seine Arbeit noch nicht veröffentlicht, als ich mit dieser Untersuchung begann. Da *Woodhouse* wie die meisten Forscher auf diesem Gebiet mit sehr kleinen Serien gearbeitet hat, ist es fast unmöglich, seine Resultate zu beurteilen, weil sie nicht statistisch bearbeitet werden können. Weil von den tumoraffinen farbstoffen Trypanblau und Blei am leichtesten in eine feste chemische Verbindung gebracht werden können, wurde diese Kombination gewählt. Das Produkt war in Wasser ziemlich schwer löslich. Die in Zimmertemperatur gesättigt Lösung enthält 10,37 mg % feste Substanz, entsprechend 4,24 mg % metallischen Blei. Sie erwies sich als wenig toxisch. 20 Mäuse, welche wöchentlich subkutan über dem Sacrum 6 Injektionen von 0,4 ml Flüssigkeit je 20 g Körpergewicht erhielten, lebten durchschnittlich ca. 5 Monate, 4 von ihnen über 8 Monate. Die am längsten lebenden Tiere bekamen eine Gesamtdosis von 6,1 mg metallischem Blei, entsprechend 305 mg je kg Körpergewicht (gleich eine Dosis von 21 g Blei für einen mittelgrossen Menschen).

Methodik.

Das Präparat wurde bei Zimmertemperatur in steriler Aqua destillata durch Schütteln gelöst und dann in einem Messkolben mit eingeschliffenem Glasstöpsel aufbewahrt. Das kristallinische Präparat, welches im Exsikkator verwahrt wurde, muss wegen der Toxizität des Bleies als steril betrachtet werden. Die Lösung wurde wöchentlich oder alle zwei Wochen erneuert. Es wurde darauf gesehen, dass sie vor der Anwendung immer klar war. Vor den Injektionen wurde 65 %iger Alkohol durch Spritze und Kanüle gesaugt. Die Injektio-

nen fanden in der Weise statt, dass ungefähr die halbe Menge auf jeder Seite des Tieres eingespritzt wurde, um die Resorption zu erleichtern und die Entstehung oberflächlicher Hautnekrosen zu verhindern. In einem Teil der Fälle bekamen die Mäuse trotzdem eine oberflächliche Hautnekrose über der Injektionsstelle. Nach etwa einer Woche trat Demarkation und Heilung ein, ohne dass der Allgemeinzustand der Tiere merklich beeinträchtigt wurde.

Die Untersuchung umfasst bisher 520 Mäuse, davon 281 Versuchs- und 239 Kontrolltiere. Das Durchschnittsalter bei Versuchsbeginn dürfte in den beiden Gruppen ungefähr 3 Monate betragen haben. Ca. 40 % der Weibchen und ca. 30 % der Männchen bekamen Tumoren, was mit *Vannfält's* Feststellung übereinstimmt, dass Weibchen häufiger durch Teerpinselung Kanzer bekommen als Männchen.

Die Methodik, die bei der Fütterung, der allgemeinen Pflege und der Teerpinselung der Tiere befolgt wurde, ist in *Vannfält's* Arbeit »Über die Einwirkung von Glykose auf Teertumoren bei weissen Mäusen« ausführlich beschrieben. Als Versuchstier diente die gewöhnliche weisse Laboratoriumsmaus. Da der Tierstamm in mehreren Sendungen von dem Lieferanten geschickt wurde, teilte ich jede Gruppe in zwei ungefähr gleich grosse Teile für Versuchs- und Kontrolltiere ein, wobei ich bestrebt war, auch die Verteilung auf die Geschlechter gleichmässig zu machen. Die Mäuse wurden von Anfang an zu je 5 in Käfigen von $30 \times 20 \times 18$ cm Grösse gehalten, die in vier Abteilungen geteilt waren. Neben einer Abteilung mit Weibchen befand sich immer eine mit Männchen. Die Käfige mit den Kontroll- und den Versuchstieren standen abwechselnd nebeneinander, damit Zug vom Fenster, Licht und andere eventuell schädliche Faktoren das Resultat nicht beeinflussen könnten. Die Tiere wurden 20 Wochen lang dreimal wöchentlich auf einer ca. $\frac{1}{2}$ cm² grossen Stelle zwischen den Schulterblättern mit Steinkohlenteer gepinselt, wobei darauf gesehen wurde, dass alle Tiere möglichst gleich viel Teer bekamen. Das verwendete Präparat trägt den Namen Saxolinteer und stammte von Fjeldhammers Brug in Norwegen.

Die Versuchszeit begann, sobald die Mäuse deutliche Warzen von Stecknadelkopf- bis Hirsekorngrösse aufwiesen. Die Versuchstiere erhielten dreimal wöchentlich über dem Sacrum eine subkutane Injektion von 0,4 ml konzentrierter Bleitrypanblaulösung je 20 g Körpergewicht. Die Versuchszeit betrug 5 Monate nach der Entstehung der Tumoren. Die wenigen Tiere, die dann noch am Leben waren, wurden getötet. Einige der Versuchstiere starben verhältnismässig bald nach Beginn der Behandlung. Bei einer Versuchszeit von 70 Tagen betrug die gesamte Behandlungsdosis ca. 15 ml, entsprechend 32 mg metallischen Blei je kg Körpergewicht. Nach den Versuchen von *Blair Bell* und anderen kann man erst nach relativ grossen Dosen einen Effekt der Behandlung erwarten. *Bell's* Patienten erhielten insgesamt 500—900 mg Blei intravenös, und erst bei den grösseren Dosen konnte er Resultate konstatieren.

Die Angabe der Entstehungszeit der Tumoren kann höchstens um einige Tage unrichtig sein, da die Tiere vor jeder Teerpinselung untersucht wurden. Da diese Fehlerquelle für Versuchs- und Kontrolltiere dieselbe ist, kann sie keine Rolle für das Resultat spielen und muss bei zunehmender Versuchszeit immer mehr an Bedeutung abnehmen.

Die toten Tiere wurden in 10 %igem Formalin gehärtet und sorgfältig obduziert. Da alle Mäuse laufend numeriert waren und nicht angegeben war, ob es sich um Kontroll- oder Versuchstiere handelte, wurden alle mit ganz dem gleichen Interesse untersucht. Hierauf wurden von allen Tumoren, Lymphdrüsen und makroskopisch verdächtigen Organen Paraffinschnitte mit Färbung nach van Gieson hergestellt. Von jedem Präparat wurden eine Anzahl Schnitte aus verschiedener Tiefe entnommen, damit keine mikroskopische pathologische Veränderung bei Untersuchung der Schnitte übergangen werden könnte. Ich war bestrebt, bei allen Präparaten mit derselben Sorgfalt zu arbeiten, was nicht schwer war, da alle dieselbe laufende Nummer hatten wie die Mäuse und nicht angegeben war, ob es sich um Versuchs- oder Kontrolltiere handelte.

Resultat.

Die Untersuchung umfasst bisher 520 Mäuse, von denen ca. 37 % Tumoren bekamen. In der ersten Zeit nach Beginn der Teerpinselung trat eine ziemlich grosse Sterblichkeit infolge von Intoxikation ein. Dies dürfte darauf beruhen, dass diese Tiere empfindlicher gegen den Teer waren, weil sie bei Versuchsbeginn nicht ganz gesund waren. Nach dem ersten Monat war die Sterblichkeit dann ungefähr gleich gross je Zeiteinheit.

Das Material ist noch nicht vollständig bearbeitet, weshalb ich bei Besprechung des Resultats nur die Tiere berücksichtige, die über 60 Tage nach Entstehung des Tumors gelebt haben. Erst nach dieser Zeit kann man ja ein Ergebnis der Behandlung erwarten, da die Mäuse vorher zu kleine Bleiden bekommen haben.

Die mittlere Versuchszeit vor der Entstehung des Tumors erhält man, indem man die Mittelwerte für die Zeit vom Beginn der Teerpinselung bis zur Entstehung des Tumors nebst ihren mittleren Fehler berechnet. Diese sind für die Kontrolltiere:

Weibchen $115,4 \pm 27,6$ (36 Tiere) Tage

Männchen $111,7 \pm 29,2$ (17 Tiere) Tage;

für die Versuchstiere:

Weibchen $122,4 \pm 22,0$ (38 Tiere) Tage

Männchen $113,3 \pm 34,7$ (22 Tiere) Tage.

Aus dieser Übersicht geht hervor, dass die Disposition zu Tumor in beiden Tiergruppen gleich gross war und also das Versuchsergebnis nicht beeinflussen kann.

Tabelle 1.

A. Anzahl überlebender Tiere am Ende jedes Versuchsmonate, berechnet in Prozent der Anzahl Tiere, welche Tumoren bekamen.

		1. Monat	2. Monat	3. Monat	4. Monat
Versuchstiere	Männchen	85,72	52,38	37,38	19,05
	Weibchen	85,96	68,42	35,26	15,79
Kontrolltiere	Männchen	91,11	77,78	51,85	22,22
	Weibchen	93,62	85,11	36,17	10,64

B. Differenz zwischen der Anzahl überlebender Kontroll- und Versuchstiere in Prozent der Anzahl Tiere, die in den einzelnen Gruppen Tumoren bekamen.

Nach dem 1. Monat	Männchen	+ 5,39 ± 8,04 %
	Weibchen	+ 7,66 ± 6,01 %
» » 2. »	Männchen	+ 25,40 ± 11,56 %
	Weibchen	+ 16,69 ± 8,29 %
» » 3. »	Männchen	+ 14,47 ± 12,09 %
	Weibchen	+ 0,91 ± 9,43 %
» » 4. »	Männchen	+ 3,17 ± 9,80 %
	Weibchen	+ 5,15 ± 6,61 %

Hieraus ist ersichtlich, dass die Sterblichkeit am Ende des zweiten Monats bei den Versuchstieren sowohl für die Männchen als für die Weibchen grösser ist als bei den Kontrolltieren und dass dieser Unterschied statistisch wahrscheinlich gemacht ist. Dies dürfte darauf beruhen, dass die toxische Wirkung des Bleies die Sterblichkeit der Tiere erhöht. Im dritten und noch deutlicher im vierten Monat besteht numerische Identität zwischen Versuchs- und Kontrolltieren. Diese Änderung könnte sich dadurch erklären, dass das Blei vermöge eines hemmenden Einflusses auf die Metastasierungstendenz der Tumoren die durch die Tumoren bedingte Mortalitätssteigerung vermindert und so die durch die Toxizität des Bleies eventuell erhöhte Sterblichkeit ausgeleicht. Das Bleipräparat hat sich ja übrigens, wie oben erwähnt, als wenig toxisch erwiesen.

Das mittlere Tumoralter, d. h. der Mittelwert nebst seinem mittleren Fehler für die Zeit, welche die Tiere nach der Entstehung des Tumors lebten, beträgt für die Tiere, die länger als 60 Tage nach der Entstehung des Tumors am Leben blieben:

Versuchstiere: $99,32 \pm 24,86$ Tage (60 Tiere)

Kontrolltiere: $97,53 \pm 28,76$ Tage (52 Tiere)

Das mittlere Tumoralter der Männchen beträgt für die Kontrolltiere $105,94 \pm 22,98$ Tage und für die Versuchstiere $105,52 \pm 26,59$ Tage.

Das mittlere Tumoralter für die Weibchen ist bei den Kon-

trofftieren $87,95 \pm 20,66$ Tage und bei den Versuchstieren $95,21 \pm 20,45$ Tage.

Die Tumoren dürften deshalb in einem bestimmten Alter bei Kontroll- und Versuchstieren im wesentlichen dieselbe Intensität der Metastasierungstendenz besitzen. Da das mittlere Tumoralter bei den beiden Tiergruppen numerisch identisch ist, hat es keinen Einfluss auf die Metastasierungstendenz zugunsten einer der beiden Gruppen.

Wenn man, um beim Vergleich zwischen Kontroll- und Versuchstieren genügend viele Tiere in jeder Gruppe zu bekommen und Gleichzeitig den Einfluss der Behandlungszeit auf die Metastasierungstendenz zu erkennen, die Tiere in zwei Gruppen mit einem Tumoralter von 61—100 bzw. 101—160 Tagen eingeteilt, erhält man folgendes Resultat:

Tumoralter 61—100 Tage: Von den Versuchstieren haben 15 von 33 Metastasen = 45,5 %, und von den Kontrolltieren haben 16 von 32 Metastasen = 50,0 %. Die Differenz zwischen Kontroll- und Versuchstieren beträgt dann $4,5 \pm 12,4$ %, d. h. es herrscht numerische Identität zwischen den beiden Gruppen. Die Behandlung hat also während dieser Zeit keine nachweisbare hemmende Wirkung auf die Metastasierungstendenz der Tumoren ausüben können.

Tumoralter 101—160 Tage: Von 20 Kontrolltieren haben 16 Metastasen, während 11 Versuchstiere von 28 Metastasen aufweisen, entsprechend 80,0 bzw. 39,3 %. Die Differenz zwischen Kontroll- und Versuchstieren ist $40,7 \pm 12,9$ %. *Die Behandlung hat also bei einer Dauer von über 100 Tagen eine statistisch sichergestellte Herabsetzung der Metastasierungstendenz bei den Versuchstieren herbeigeführt, die über dreimal so gross ist als ihr mittlerer Fehler.*

Will man das Material daraufhin untersuchen, inwieweit das Geschlecht einen Einfluss auf das Behandlungsergebnis hat, so muss man alle Tiere mit einem Tumoralter über 60 Tage mitrechnen, um genügend viele Tiere in jeder Gruppe zu bekommen. Bei diesem Verfahren erhält man natürlich ein schlechteres Ergebnis, als wenn man nur Tiere mit einem Tumoralter über 100 Tage berücksichtigen würde.

Tabelle 2.

Tabelle über die Anzahl Tiere, die innerhalb eines Zeitraums von 10 Tagen seit der Entstehung des Tumors starben. Männchen und Weibchen sind in jeder Gruppe getrennt aufgeführt. Die Tabell gibt an, ob die Tiere, welche sämtliche histologisch festgestellte maligne Tumoren haben, an einer oder mehreren Stellen (Lymphdrüsen, Lungen, Leber oder Nieren) Metastasen aufweisen oder nicht.

Kontrolltiere.

Tumoralter in Tagen	61— 70	71— 80	81— 90	91— 100	101— 110	111— 120	121— 130	131— 140	141— 150	151— 160
Geschlecht	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀
Tiere ohne Metastasen	— 6	2 4	— 3	— 1	— 2	— 1	— —	— —	— 1	— —
Tiere mit einer Metastase	— 2	1 3	1 —	1 1	2 1	— 3	2 —	1 —	1 —	— —
Tiere mit 2 oder mehr Metasta- sen	— 1	1 —	— 3	2 —	— 2	1 —	1 1	1 —	— —	— —
Anzahl Tiere in jeder Gruppe	— 9	4 7	1 6	3 2	2 5	1 4	3 1	2 —	1 1	— —

Versuchstiere.

Tumoralter in Tagen	61— 70	71— 80	81— 90	91— 100	101— 110	111— 120	121— 130	131— 140	141— 150	151— 160
Geschlecht	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀
Tiere ohne Metastasen	2 4	3 2	— 4	— 3	3 3	1 —	1 4	4 —	— —	1 —
Tiere mit einer Metastase	1 1	— 3	1 1	1 2	1 1	— 1	1 2	— 1	— 1	— —
Tiere mit 2 oder mehr Metasta- sen	— 1	— 1	— 1	1 1	— 1	— —	1 —	— 1	— —	— —
Anzahl Tiere in jeder Gruppe	3 6	3 6	1 6	2 6	4 5	1 1	3 6	4 2	— 1	1 —

Tumoralter über 60 Tage:

Weibchen: Von den Kontrolltieren haben 17 von 35 Metastasen = 48,8 %, während 19 Versuchstiere von 39 Metastasen haben = 48,6 %. Die Differenz zwischen Kontroll- und Versuchstieren ist $0,2 \pm 11,6$ %. *Die Behandlung hat also keinen hemmenden Effekt auf die Metastasierungstendenz der Tumoren bei den Weibchen gehabt.*

Männchen: Von den Kontrolltieren haben 15 von 17 Metastasen, die entsprechenden Ziffern bei den Versuchstieren sind 7 von 22; die Prozentzahlen sind 88,2 bzw. 31,2 %. Die Differenz ist $57,0 \pm 12,7$ %. *Die Behandlung hat also eine statistisch sichergestellte Herabsetzung der Metastasierungstendenz der Tumoren bei den Männchen um mehr als das Vierfache ihres mittleren Fehlers zur Folge gehabt.*

Nach diesem Resultat kan man erwarten, dass die Behandlung mit Bleitrypanblau auch die Neigung zu gleichzeitiger Metastasierung an mehreren Stellen im Organismus vermindern wird, d. h. dass die Behandlung die Malignität der Tumoren überhaupt herabsetzt. Bei Weibchen mit Metastasen an zwei oder mehr Stellen besteht numerische Identität zwischen Kontroll- und Versuchstieren, während man bei den Männchen möglicherweise einen Unterschied ablesen kann. Während 35,7 % der Kontrollmännchen Metastasen an zwei oder mehr Stellen aufweisen, ist dies bei den Versuchsmännchen nur in 9,1 % der Fall. Die Differenz ist $26,3 \pm 13,1$ %, also nur statistisch wahrscheinlichgemacht. Dies beruht darauf, dass das Material für diese Berechnung zu klein ist.

Um zu sehen, ob die Behandlung einen Einfluss auf das Wachstum der Primärtumoren hat, habe ich das mittlere Tumorwachstum je Tag in mg für Tiere mit einem Tumor von über 100 mg Gewicht berechnet. Diese Mindestgrösse wählte ich um allzu grosse Fehler beim Ausschneiden und Wägen zu vermeiden. Das Tumorwachstum wurde je Tier und Tag von dem Tage, an dem der Tumor entdeckt wurde, bis zum Tode des Tieres berechnet. Hierauf wurde der Mittelwert mit seinem mittleren Fehler für sämtliche Tiere in den verschiedenen Gruppen berechnet. Dabei ergaben sich folgende Werte:

	Mittleres Tumorwachstum je Tag	
Versuchstiere	{	Männchen $20,47 \pm 14,41$ mg (25 Stück)
	{	Weibchen $22,25 \pm 21,94$ mg (35 Stück)
Kontrolltiere	{	Männchen $44,44 \pm 58,19$ mg (19 Stück)
	{	Weibchen $33,79 \pm 29,93$ mg (34 Stück)

Hieraus geht hervor, dass die Männchen und Weibchen der Kontrolltiere nur ein numerisch grösseres mittleres Tumorwachstum je Tag haben als die Männchen und Weibchen der Versuchstiere. Dies beruht möglicherweise darauf, dass das Material für diese Berechnung zu klein ist. Man kann also keine Schlüsse ziehen, ob die Behandlung einen hemmenden Einfluss auf das Tumorwachstum der Primärtumoren ausgeübt hat oder nicht.

Diskussion.

Eine makroskopische Besichtigung der Versuchstiere zeigt, dass das subkutane Gewebe an der Injektionsstelle stark blaufärbt ist. Farbstoffe in reichlicher Menge finden sich ferner in der Leber und Milz sowie, wenn auch nicht so viel, in den Nieren, Nebennieren und Lymphdrüsen. Dagegen ist nur in einige Fälle eine leichte Blaufärbung der Primärtumoren zu entdecken. Durch die chemische Bindung an Blei verliert also Trypanblau seine Tumoraaffinität. Die hemmende Wirkung von Bleitrypanblau auf die Metastasierungstendenz könnte darauf beruhen, dass es vermöge der Toxizität und des allgemeinen gärungshemmenden Einflusses des Bleies durch sein Vorhandensein in den Lymphdrüsen und anderen Organen verhindert, dass metastatische Tumorzellenverbände in diesen wachsen.

Auch ein geschlechtgebundener Faktor ist mit im Spiele, da Bleitrypanblau nur bei den Männchen hemmend auf die Metastasierungstendenz wirkt. *Vannfält* fand, dass bei einem Tumoralter von 61—150 Tage bei den Kontrollweibchen eine grössere Tendenz zu Krebsmetastasen in den regionalen Lymphdrüsen bestand als bei den Kontrollmännchen und dass dieser Unterschied zwischen den Geschlechtern statistisch

sichergestellt war. In meinem Material tritt nur ein numerischer hervor, und zwar zugunsten der Männchen (die Differenz zwischen den Männchen und den Weibchen der Kontrolltiere betrug $16,0 \pm 13,8 \%$). Dies spricht nicht gegen *Vannfält's* Resultat, sondern beruht darauf, dass mein Material für diese Berechnung zu klein ist.

Dass das Geschlecht mit seinen speziellen Hormonen Einfluss auf die Wachstumstendenz des Kanzers hat, steht ausser Zweifel. Bis jetzt liegen jedoch hauptsächlich Arbeiten vor, welche für oder gegen die kanzerogene Wirkung der weiblichen Geschlechtshormone sprechen. So fanden *Burns, Suntzeff & Loeb, Gilmour, Zondek; Gardner, Smith, Strong & Allen* u. a., dass weibliche Geschlechtshormone, in relativ grossen Dosen während längerer Zeit, Tumoren hervorzurufen oder auch nur die Malignität vorhandener Tumoren zu steigern vermögen. Andererseits sind *Bischoff & Maxwell, Butenandt* sowie *Dodds* der Ansicht, dass weibliche Geschlechtshormone keine kanzerogene Wirkung ausüben. *Schockhaert* glaubt festgestellt zu haben, dass Gravidität das Wachstum von Kanzer begünstigt, während *Farati* entgegengesetzter Auffassung ist. *Heiman & Krehbiel* fanden, dass Kastration bei Männchen das Wachstum von Impftumoren steigert, während Kastration bei Weibchen die entgegengesetzte Wirkung hatte. Dies soll dafür sprechen, dass die normal vorkommenden Hormone einen gewissen Einfluss auf das Kanzerwachstum haben, wobei männliche Hormone hemmend, weibliche fördernd wirken. Ferner kann sich *Vannfält's* Resultat bei Normalmaterial hierdurch erklären.

Die vorliegende Untersuchung hat gezeigt, dass die Behandlung mit Bleitrypanblau einen statistisch sichergestellten hemmenden Einfluss auf die Tendenz der Tumoren hat, Metastasen in den Lymphdrüsen von Männchen zu setzen. Bei den Weibchen hat die Behandlung dagegen keinen nachweisbaren Effekt gehabt. Bei den Männchen wirkten die Geschlechtshormone hemmend auf die Metastasierungs-tendenz, welche Wirkung durch die Bleitrypanblauhandlung so verstärkt wurde, dass der Unterschied zwischen behandelten und

unbehandelten Tieren statistisch sichergestellt werden konnte. Dagegen besteht bei den Weibchen numerische Identität. Ihre Geschlechtshormone haben nach *Agduhr* bei Weibchen, welche getrennt von Männchen leben, zur Folge, dass die Weibchen grössere Widerstandskraft gegen verschiedene Gifte besitzen als die Männchen unter entsprechenden Bedingungen. Es wäre daher denkbar, dass die weiblichen Geschlechtshormone eine entgiftende Wirkung auf Bleitrypanblau ausüben, wodurch der hemmende Einfluss der Behandlung auf die Metastasierungstendenz herabgesetzt werden würde. Es wäre ja möglich, dass die Behandlung auch bei den Weibchen einen nachweisbaren Erfolg erkennen lassen würde, wenn man mit einem wesentlich grösseren Material arbeiten würde.

Zusammenfassung.

Der Verfasser hat die Wirkung von Bleitrypanblau auf den durch Teerpinzelung bei weissen Mäusen hervorgerufenen Tumor untersucht. Das Präparat wurde den Tieren durch subkutane Injektionen zugeführt. Die Behandlung hemmte die Tendenz der Tumoren, Metastasen in den regionalen Lymphdrüsen von Mäusen zu setzen, die mindestens 100 Tage lang behandelt wurden, und der Unterschied zwischen den unbehandelten und den behandelten Tieren war um mehr als das Dreifache seines mittleren Fehlers statistisch sichergestellt. Dieses Resultat beruht ausschliesslich auf den Männchen, bei denen der Hemmungseffekt statistisch sichergestellt ist, da die Differenz in der Metastasierungsfrequenz zwischen behandelten teergepinselten Mäusenmännchen und teergepinselten, aber im übrigen unbehandelten Mäusenmännchen ihren mittleren Fehler um mehr als das Vierfache übersteigt.

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STUDIES ON HEREDITARY DWARFISM IN MICE III.*)

DEVELOPMENT OF THE ADRENALS IN DWARF MICE.**)

By *Erik D. Bartels*.

(Received for publication March 1st, 1940).

With the object of attempting to define the comparative conditions between the adrenals of hypophyseal dwarf mice and those of normal mice, a number of adrenals of dwarf and of normal mice of different ages varying from one week to eighteen months have been examined. The literature of this subject shows that the adrenals of mice undergo a peculiar development, different in the two sexes, that does not appear to occur in any other kind of animal. Mention will be made later of certain conditions in human beings that have been considered, though certainly erroneously, to be analogous.

The development referred to take place at the border region between the medulla and the cortex where, on the site

*) Previous publications in this series:

I. Kemp, T. und Marx, L.: Beeinflussung von erblichem hypophysärem Zwergwuchs bei Mäusern durch verschiedene Hypophysenauszüge und durch Thyroxin. *Acta path. et microbiol. scand.* XIII, 1936, 512 und XIV, 1937, 197.

II. Kemp, T.: Heredity and the endocrine function. An Investigation of Hereditary Pituitary Deficiency in the Mouse. *Acta path. et microbiol. scand. Suppl.* XXXVII, 1938, 290.

**) Given as a lecture in slightly altered form to The Biol. Soc. Copenhagen, 14—2—1940.

of the zona reticulata, a particular zone is first formed, called in the literature the X-zone, which subsequently disappears and is replaced by a thicker or thinner layer of connective tissue. On the cortex side of this a very narrow zona reticulata is often formed.

The development was first mentioned by Masui and Tamura, and was later described in detail by Howard-Miller, Deanesly, Cramer, Waring, and by Grollman, to whose work I have unfortunately not had access. Howard-Miller's description is the most extensive and is also that which agrees best with my findings for which reason it will be referred to in the following. Deanesly is also in close accord with it, and Waring, (who gives the most detailed record of the cytological features), whilst Cramer is of a somewhat different opinion.

Before these features are described more fully, a very short review of the structure of the adrenals of mice will be given. The organ has approximately the shape of an oblate spheroid, varying, however, from spherical to kidney shape. Its size in an adult mouse is about that of a large pin's head. It has not been weighed because the difficulty of isolating it completely from the surrounding fat involves too great uncertainty. It is enclosed by a fairly strong capsule of connective tissue. The medulla is collected in the middle and is easily distinguished from the cortex in preparations stained with hematoxylin-eosin, because it appears yellow in contrast with the cortex's red. Apart from small irregularities in the transitional region medullary tissue is never seen outside the central compact mass.

The cortex consists external of a zona glomerulosa which, in animals one week old, comprises about half the cortex. After this period it gradually decreases so that from the third week it only consists of a single row of groups of cells, each comprising from four to eight cells. These are arranged in small rounded bodies and have oval nuclei fairly rich in chromatin and a cytoplasm that is rather sparse, though light, and often contains single enclaves, as do the cells in the zona fasciculata. There is no definite boundary between the latter

and the glomerulosa, and in most stages it comprises $\frac{2}{3}$ to $\frac{3}{4}$ of the cortex. It is not yet completely differentiated in the one-week-old mouse because the cells on its site are apparently arranged quite unsystematically; but differentiation is evident even from the second week, after which no further change occurs. The cells are deposited in radial columns, sometimes appearing to consist of a single row of cells and sometimes of two or three rows. Lengthy capillaries are found between these rows. The cells have large, oval nuclei with rather less chromatin content than those in the glomerulosa. The cytoplasm is abundant and is blistered all over with small vacuoles, the enclaves containing lipid.

Central to this zone, in older animals, two or three rows of small cells are found, frequently with somewhat edged nuclei rich in chromatin and a little dark cytoplasm. The arrangement is irregular and numerous irregular capillaries are found. A more or less thick layer of connective tissue can be seen in the region bordering the medulla. In young animals, on the other hand, a transition layer is found which undergoes a characteristic development that will be more fully described later on. Presumably it would be reasonable to call the narrow layer in the old animals the zona reticulata, as is usual in the case of other kinds of animals. I shall, however, call the transition layer in young animals the X-zone, as previous authors have done.

The medulla is composed of large polygonal cells with large, bladder-shaped nuclei containing but little chromatin. The cytoplasm appears to be amorphous and yellow when stained with hematoxylin-picrosesosin; but, especially in older animals, two types of cells are often seen, one with a more reddish colouring of the cytoplasm, the other with a more yellow colouring. They lie apart in small groups.

The arrangement of the cells in the young animals is trabecular with cell columns crossing each other and enclosing large cavities filled with blood. The cells in the old animals, however, are in spherical groups, each enclosed by a thin layer of connective tissue and with, literally, no ca-

vities between them. The cells in each group appear to be arranged radially.

This development of the medulla seems to occur whilst the boundary layer between the medulla and the cortex is being formed; but its progress is not always quite parallel with this development, as I have noticed medulla of this

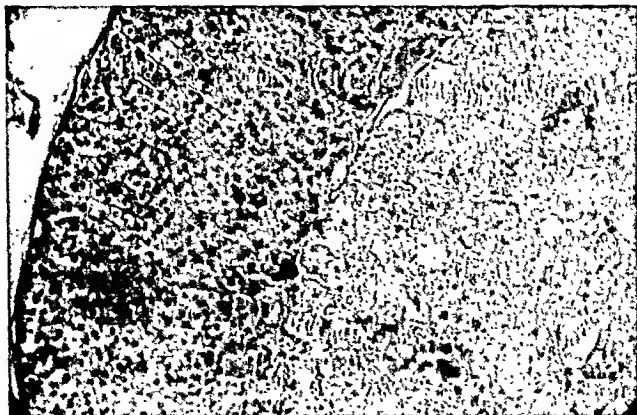


Fig. 1.

older type in a couple of animals even before the connective tissue was found between the medulla and the cortex.

As has already been mentioned, the transitional region between the medulla and the cortex displays special features during the growth and maturing of the animal. What these peculiarities are will be best understood by following the development of the organ.

Fig. I (hem.-picroeosin, approx. 50/1) shows the adrenal of a male aged 14 weeks, a period at which the development of the male is complete. The cortex is seen to be clearly defined from the medulla by a layer of connective tissue. The zona glomerulosa is quite narrow, and the zona fasciculata forms the greater portion of the cortex. The last two or three rows of cells in towards the layer of connective tissue are somewhat irregular and form a narrow zona reticulata.

Fig. II (hem.-picroeosin, approx. 250/1) shows the region of transition between the medulla and the cortex of a male aged

seven days. At this stage the development of the adrenals in males and females is identical. The innermost cells of the comparatively large zona glomerulosa are seen, the more loosely organized layers, and, within these, the transition from the cortex to the medulla. This transition is quite gradual without any boundary line whatever. The medullary fibres extend towards the cortex and meet off-shoots from it, so that strings of cells are formed one end of

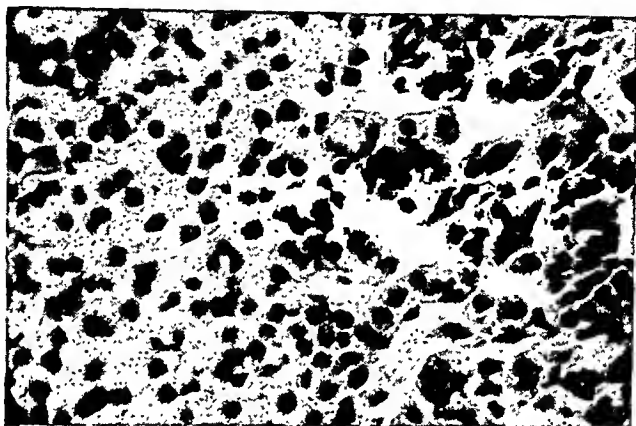


Fig. 2.

which consists of cortical cells whilst the other is of medullary cells. Where the one tissue begins and the other ends can only be demonstrated by their susceptibility to the stains.

The manner in which the zona fasciculata is formed in both sexes then gradually becomes apparent; but a special layer is simultaneously formed in the transitional region between the medulla and the cortex, its cells being comparatively small, with sparse darkish cytoplasm, and its nuclei rich in chromatin. The transition to the medulla is still quite imperceptible. This development is especially rapid in the females, in which the layer mentioned may become half as thick as the cortex, whilst it never attains any great size in the males. The so-called X-zone can already be discerned in ten-day-old animals and is the same for both sexes until they are about 21 days of age, after which the development differs in accordance with sex. As an example of these stages, a section of the adrenal of a 21-day-old female is shown in *fig. III* (hem.-picroeosin, approx. 250/1). The layer in question is seen with its rather small dark cells and the almost imperceptible transition from the cortex to the medulla. I have

never seen the male X-zone thicker than in this specimen (i. e. five or six layers of cells). It now begins to disappear in this sex, a process which seems to take place quite imperceptibly. Possibly the nuclei become slightly darker and oblong connective tissue nuclei appear in different parts of the layer. These are

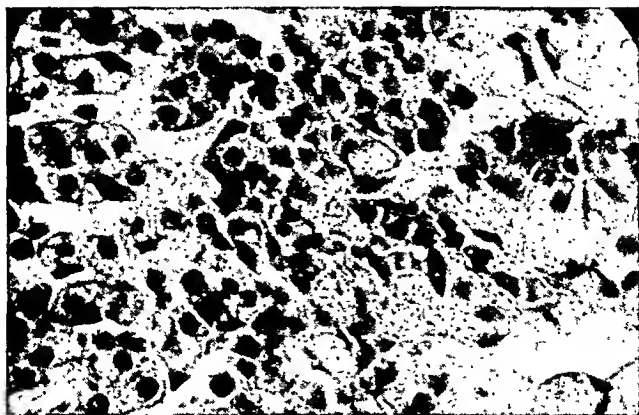


Fig. 3.

situated neither on the border between the medulla and the cortex nor on the border between the X-zone and the rest of the cortex. They seem to be distributed in the X-zone. The connective tissue increases in volume and the true X-zone cells disappear, thus causing a definite boundary between the medulla and the cortex

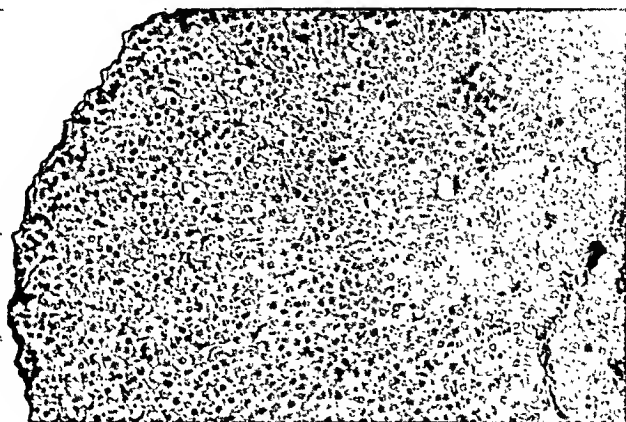


Fig. 4.

marked by a thicker or thinner layer of connective tissue. Finally, the innermost cell layers are organized in the zona fasciculata as a narrow zona reticulata, the resultant picture being as that shown in fig. I.

On the other hand, in the female the X-zone continues to grow.

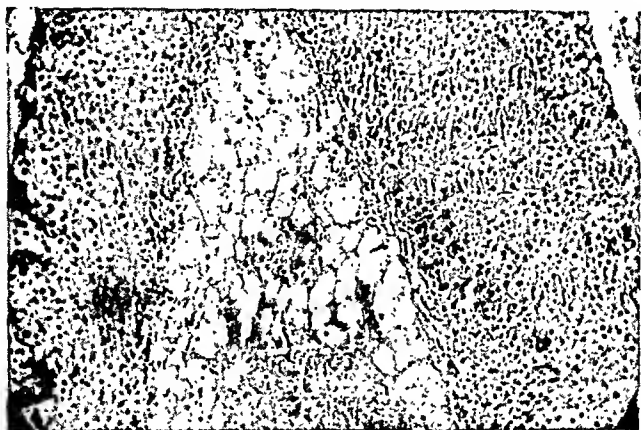


Fig. 5.

A preparation from a five-week-old female is seen in *fig. IV* (hem.-picroeosin, approx. 50/1). This shows that the X-zone forms between $\frac{1}{3}$ and $\frac{1}{4}$ of the whole cortex and that here, too, the transition from the cortex to the medulla is quite imperceptible.

After a few weeks the X-zone begins to degenerate and in

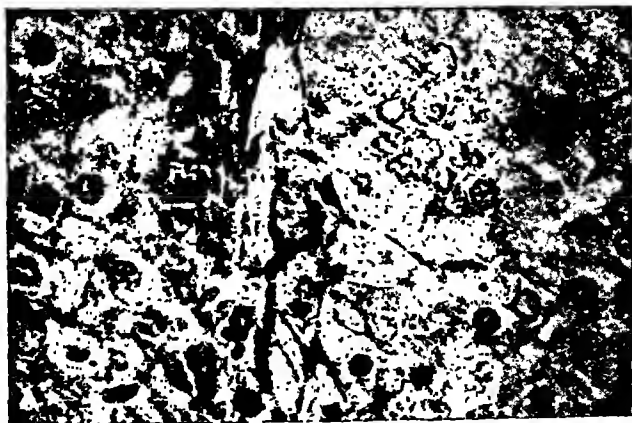


Fig. 6.

the female this process is very violent. There is hyperaemia and the nuclei disappear. Vacuoles are formed in the cells and, spreading throughout the cell, subsequently merge with neighbouring cells so that this region is reminiscent of normal fatty tissue. At the same time, a thicker or thinner layer of connective tissue forms at the transition from the zona fasciculata to the X-zone. *Fig. V* (hem.-picroeosin, approx. 50/1) shows the culmination of the process in a 15-week-old female. The degenerated X-zone diminishes gradually, a zona reticulata generally being formed in the same way as in the male, and at length it is impossible to distinguish between male and female adrenals. The final stage in the female is seen in *fig. VI* (hem.-picroeosin, approx. 250/1).

As has already been stated, this description agrees fairly well with that given by Howard-Miller, although she examined a much greater number of animals and is, therefore, in a position to make more definite reports regarding time and variations. She states that the X-zone is first seen in both sexes at the age of ten days, culminates in the male at the age of three weeks, ususally begins to degenerate at the age of four weeks, and has disappeared, without exception, at the age of six weeks. If a young male be castrated, the process develops quite differently, actually as in the females. The administration of androgenes after castration causes the process to take a normal course. The X-zone continues to grow in the females until the age of four or five weeks is reached, when it may form as much as half the cortex. Sooner or later, it begins to degenerate. Degeneration set in at the age of 50 days in half the number of mice examined, and in all of them by the 93rd day. The final stage is reached in from 80 to 200 days. The degeneration takes place either as shown in the photographs or without formation of vacuoles; the latter is by far the more rare. Castration does not alter the process; pregnancy and partus accelerate it.

Howard-Miller was unable to prove any definite connection with adolescence. The males may be fertile before degeneration of the X-zone commences, and the females are very frequently fertile long after it has been completed. To elucidate the question, the card-index for our mice has been checked

for two periods chosen at random. Breeding is carried out on the principle that out of each litter one male and all the females are retained and allowed to live together until all the females become pregnant. The first pregnancy may thus be considered to be an indication of the age of maturity both of males and females. *Fig. VII* shows a curve of the time of

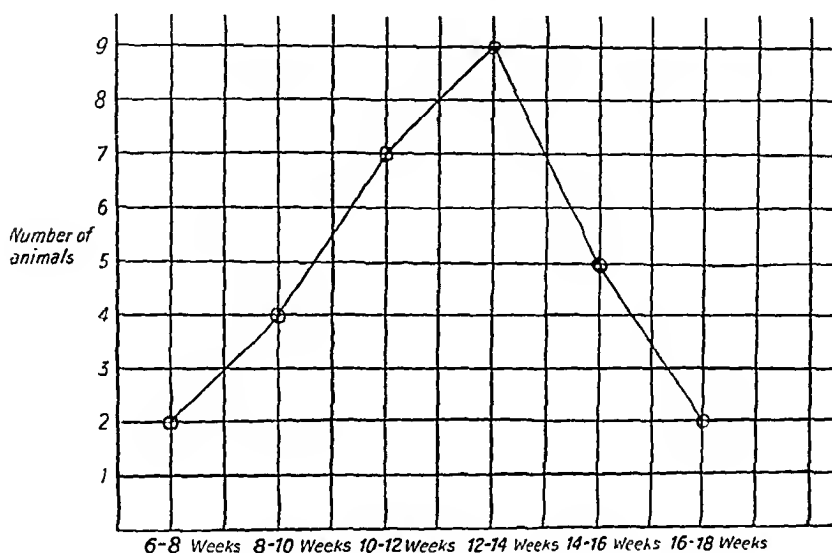


Fig. 7.

occurrence of the first birth for 31 female mice. It will be seen that the result is a fairly even curve of distribution culminating in about the twelfth or fourteenth week. If the period of pregnancy of the mouse, three weeks, be subtracted, it becomes evident that at least two males were fertile whilst the X-zone existed. Table I shows how old 48 females were at the last partus before they died or were killed. It will be seen that several are over the 6—8 months set as the ultimate limit for the total disappearance of the X-zone.

Howard-Miller is of the opinion that the X-zone produces substances that are at any rate related to the male sex-hormone. In support of this she points out that the changes in the secondary sex characteristics of castrated male mice with

Table I.

4— 6 Months:	7 animals.
6— 8 »	: 19 »
8—12 »	: 17 »
12—18 »	: 5 »

Age of 48 female mice at the time of their last birth.

the X-zone are much smaller than those of rats, which have no X-zone. Cramer considers that the X-zone is formed from the medulla. He bases this opinion partly on the formation of connective tissue between the X-zone and the rest of the cortex, and partly on the deposition of the cells close in to the medulla. Here he thinks he observed medullary follicles consisting partly of cells containing adrenalin — chromaffine cells — and partly of transitional forms and X-zone cells. He furthermore holds that the X-zone does not produce androgenous substances, but that it is displaced by androgens. That it also disappears in female mice might be due to the fact that they form androgenous substances at a later age.

Together with Howard-Miller and Deanesly I must, however, insist that the X-zone clearly develops from the cortex. This is shown both by its very beginning and by its quite even development by stages as shown in the pictures. Furthermore, Waring, in studying the embryological and early post-natal development of the adrenals, has clearly confirmed this.

Deanesly, though, is also of the opinion that the X-zone does not form and is not particularly dependent on androgens. She supports her theory partly by her observations of hypophyseal dwarf mice obtained from the strain belonging to The University Institute for Human Genetics. My observations agree entirely with hers, for which reason I am able to illustrate them by a couple of pictures.

As has already been demonstrated by Kemp and Marx, the adrenal cortex in hypophyseal dwarf mice is very narrow, whereas the medulla does not seem to deviate from the normal, at any rate when the weight of the animal is taken into consideration. The more detailed structure of the cortex is not dealt with in that study.

In agreement with Deanesly I now find from my examina-

tion of a series of the adrenals of dwarfs aged from fifteen days (the earliest age at which it has hitherto been possible to diagnose the abnormality by external examination) to about eight months, that the cortex, in spite of its slight

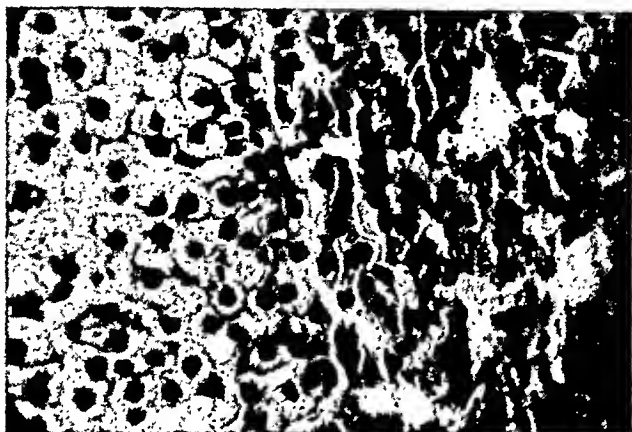


Fig. 8.

quantitative development in both sexes behaves exactly as the cortex of the normal male.

The preparation made from a fifteen-day-old dwarf can hardly be distinguished from a normal one. *Fig. VIII* (hem.-picroeosin, approx. 250/1).

Fig. IX (hem.-picroeosin, approx. 250/1) shows a four-week-old

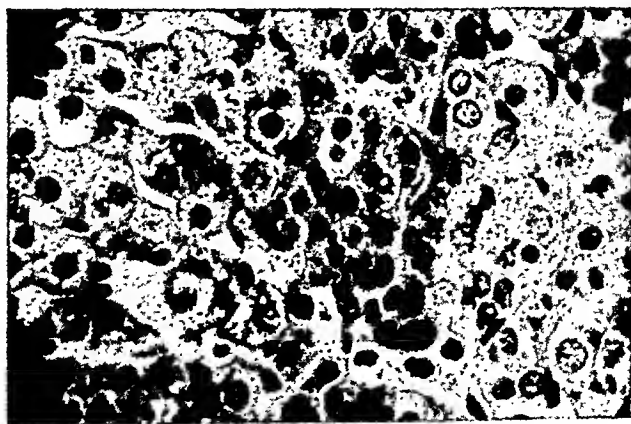


Fig. 9.

female. All the zones are seen to be well developed including the X-zone; but connective tissue nuclei are seen in certain places in the X-zone already at this age.

Fig. X (hem.-picroeosin, approx. 250/1) shows a female aged nine weeks. The process is complete. The cortex is separated from

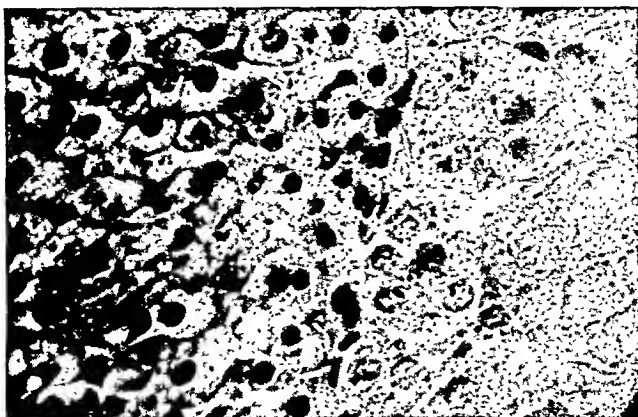


Fig. 40.

the medulla by a layer of connective tissue that is clearly visible.

Fig. XI (hem.-picroeosin, approx. 250/1) is included in order to show how thin the cortex can be in the old dwarfs, whilst at the same time all the zones are distinctly visible. This picture was obtained from a female aged 32½ weeks (2).

According to Deanesly, the castration of males causes no alterations in this respect.

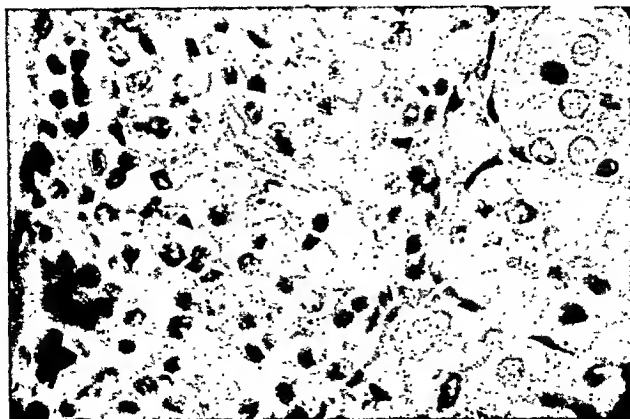


Fig. 41.

Judging from these discoveries, the X-zone might probably be expected to be dependent on the hypophysis, since it can hardly be supposed that female dwarfs would produce a particularly large quantity of male sex-hormone and thus cause the X-zone to disappear earlier than in normal females. On the other hand, Deanesly does not believe that it can be the gonadotrophic hormones that exert their influence, since Smith and McDowell have shown that at any rate they are produced in sufficient quantity to allow a certain development in the sexual glands. Against this argument the objection can, of course, be raised that the gonadotrophic hormones are, however, undoubtedly greatly reduced in quantity, and that this reduction may possibly be sufficient to disturb the normal development of the X-zone in the dwarf females.

Quite a different possibility is that the peculiar conditions of the X-zone obtaining in the case of the dwarfs may be connected with the unquestionable change in the hypophyseal hormones that affect the metabolism and are found in these dwarfs. Dr. Møllenbach, however, will describe these hormones later on in this series.

Attempts have been made to suggest that the X-zone is analogous with the portion of the zona reticulata that degenerates in human beings during the first year of life. This, however, does not seem to be very probable. Firstly, the process occurs much later in the life of the mouse, in the case of the males first about the age of puberty, in the females during the period of sexual maturity. Secondly, according to Kohno (quoted from v. Lucadou) a connective tissue septum is found between the medulla and the cortex of human beings at birth. This disappears in the course of the first two years of life, just the reverse of what is observed in the case of the mouse. Finally, it must be noted that Howard-Miller was unable to find any analogy from her examination of monkeys.

In conclusion, mention must be made of a new theory regarding the structure and function of the adrenal, which,

should it prove correct, will be interesting, particularly if it is regarded in relation to the processes in the mouse adrenal. In his attempt to elucidate the relation between the function of the medulla and that of the cortex v. Lucadou carried out some very careful studies of the structure of the human adrenal and of that of the adrenal of different kinds of animals. He prepared wax models showing the most minute details, by means of which he came to the conclusion that both the medulla and the cortex are constructed as glandular ducts that develop in a perfectly definite characteristic manner and are interconnected. According to his finding, the adrenal, like the kidney, should be constructed of units which he suggests calling epinephrones. Where the canal ends is not stated. In accordance with this theory the cortex should be a holocrine secreting gland of which the secretion goes into the medulla where it becomes mixed with the medullary secretion, subsequently entering the blood. The co-operation between the cortex and the medulla thus becomes much more intimate than has been hitherto supposed.

So far as I can see, it is difficult to decide at present whether the theory is correct or not, because the evidence in v. Lucadou's article is not sufficiently convincing. The conditions in mice, referred to in the foregoing, rather tend to refute it as regards this kind of animal. There can be no doubt that the medulla and the cortex are dissociated when the X-zone has disappeared and the layer of connective tissue has been formed. If the theory could be proved to be correct for other animals, the process in the mouse adrenal would become doubly interesting and might possibly make the mouse particularly useful as an object for study in investigations of the adrenals.

Finally, mention much be made of a condition which is of some importance in determining the question of the æthiology of the dwarf growth in this strains of mice.

When rats are subjected to hypophyseal ectomy, there is disclosed, as first shown by Reiss, a sudanophobe cell-zone

at the border between the cortex and the medulla. In his experiments in cutting through the hypophyseal stalk, Westmann makes use of this condition as an indicator to elucidate the question whether the operation has caused complete degeneration of the hypophysis or has successfully achieved its desired result.

In the mouse the condition is complicated by the X-zone being sudanophobe. The other cortex cells in the normal animal are very strongly susceptible to Sudan colouration.

The condition in the dwarfs appears to be that occasionally, when the X-zone has disappeared, a narrow cortex zone can be seen, which, although not completely sudanophobe, is less susceptible to Sudan colouration than the outer parts of the cortex.

The phenomenon is, however, not seen in all animals, and the discovery must probably be explained by considering that the dwarf in this respect behaves as though subjected to partial hypophyseal ectomy.

Summary.

A review is given of the structure of the adrenals of normal and dwarf mice. The development and regression of the X-Zone is described. In both sexes it is first seen at the age of ten days. In the males it is only little developed and degenerates at the age of four to six weeks. In the females the X-Zone keeps on growing until the age of four to six weeks, when it may form as much as half the cortex. Later on a very violent process of degeneration is seen, and at the age of 93 to 200 days the X-Zone has totally disappeared. In the dwarf mice the X-Zone of both sexes behaves as the X-Zone of the normal male. Judging from this it might be expected, that the X-Zone is dependent of the hypofysis.

In the dwarf mice a partially sudanophobe zone is occasionally seen in the inner part of the cortex. This phenomenon must probably be explained by considering that the dwarf behaves as though subjected to partial hypophyseal ectomy.

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About the Dwarf mice in general see further publications in this series.

FROM THE ANATOMICAL INSTITUTE OF THE UNIVERSITY OF
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EXPERIMENTAL STUDIES ON THE NORMAL AND PATHOLOGICAL HISTOPHYSIOLOGY OF THE PROSTATE GLAND.

I. OBSERVATIONS IN NORMAL TEST ANIMALS BEFORE AND AFTER COPULATION.

By *P. Gylling*.

(Received for publication April 1st, 1940).

I am deeply indebted to the late Professor *Väinö Lassila*, former director of the Anatomical Institute, for the great interest he always showed in these investigations. To his successors, Dr. *Göran Hjelman*, associate professor of anatomy, and Docent *Niilo Pesonen* I beg to express my sincere thanks for the courtesy they have shown me. I should also like to acknowledge my great debt of gratitude to the late Professor *Birger Runeberg*, to Professor *Axel Wallgren*, Dr. *Johannes Wahlberg* and Dr. *Martti Mustakallio* for their advice and ungrudging assistance which have been a great help to me in my work. — Some of the expenditures entailed in this work were paid by a contribution from Kommerserådet *Otto Malm's* donation-fund.

As the experiments made in this investigation were performed exclusively on rabbits, it should be pointed out that the terminology adopted in this, as in the following, works on the prostate gland is based on *Leydolph's* (1930) investigations. I take the liberty to borrow from this author's paper the adjoining illustration which is meant to explain the interrelation of the accessory sexual glands in the rabbit. I also refer to a previous exposition of the origin and nomenclature of these organs (*Gylling* 1938).

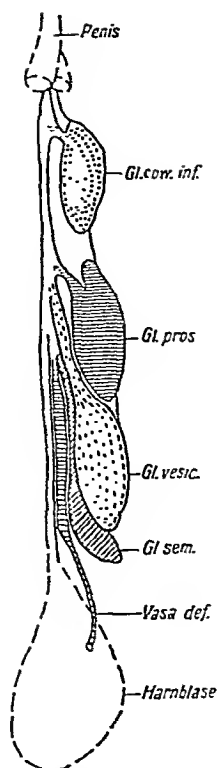


Plate 1.

The question as to the function of the prostate has come very much to the fore ever since it was observed that this organ was not exclusively made up of a cluster of muscle-fibres. It was noted that the muscular layers surrounded cavities which were lined with a typical glandular epithelium. *Stilling* (1884) was the first to observe that this epithelium underwent noticeable changes after copulation in rabbit. *De Bonis* (1907) made the same observation in dogs. However, since *Petersen* (1909) made his tests with copulation and pilocarpin injections in rabbits, no further histological experiments on this aspect to the function of the prostate gland have been published so far as I know.

As the histophysiological technique has made consider-

able progress since that date and the conception of cytologic phenomena has become essentially modified, it seems that the time has now come to add to the investigations made by earlier workers.

Since it has been shown that the prostate in the sexmature individual is in a state of continuous secretion (*Stieve* 1930), we cannot base such studies on the prostate gland from no matter what animal, since the cells of the prostatic epithelium might be in the most dissimilar stages of activity.

Burchardt (1931) has shown, however, that if the secretory activity of the testes was constant, another secretion-regulating factor developed in the *seminal vesicle* in the form of the pressure exerted on the walls of the latter by the secretion accumulated in the lumen. When the gland had attained a certain degree of fullness, this pressure had an inhibitory effect on the secretion, with the result that the epithelium retrograded and its height decreased. If the gland was emptied during copulation, this pressure disappeared, and the secretion of the epithelial cells again set in. In an investigation on the seminal vesicle and another accessory sexual gland I was able to check these observations by histophysiological methods. — Owing to the close affinity between these accessory sexual glands and the prostate, it might easily be imagined that a similar stage of inactivity, or perhaps rather of secretion preparedness, might also be created in the prostate gland, if only similar methods were applied.

Departing from this stage, a secretion would be produced if the test animals were made to copulate. As was pointed out above, the possibility of eliciting a secretory activity by copulation has already been shown in principle. In this connection the interest is focussed on the question as to whether the secretion is produced at the very moment of ejaculation when the lumen of the prostate is emptied of its secretion. Copulation *per se* does not take place in such a short period as would be desirable in determining the actual moment at which the secretion begins.

A series of preparations taken at different intervals after

copulation would give a clear picture of the secretory function of the prostate gland. It would be possible to observe whether the activity of the gland takes place during a single working cycle, or whether the function of the gland is more complicated and composed by several periods of activity.

In order as far as possible to imitate conditions in man, the rabbit was selected as test animal, since this animal has no typical periods of mating. Another circumstance which makes the rabbit very suited for such experiments is that it is very easy to determine the moment of ejaculation in this animal.

We also get an excellent opportunity of making secretion-physiological observations of a general nature. The method usually adopted in such experiments, as described by *Heidenhain* (1907), consists in producing the secretory activity either by stimulation of the secretory nerve, by injections of secretion-promoting substances or by changes in the diet. In the present investigation, however, the secretory activity could be produced without artificial means in a perfectly »natural« manner. Moreover, this secretion could be set going at a given moment, independent of the individual. If the test animals are carefully standardized, this secretion ought further to be of similar intensity and duration in the different animals. The result obtained in this manner thus would in some measure be complementary to the results obtained in experiments on other glands and by other methods.

The question presented above may be summarized as follows:

- I. Is it possible to inhibit the secretory activity in the prostate directed towards the lumen by a repletion of the lumina of the glandular ducts.
- II. What influence is exerted by copulation, and particularly by ejaculation, on the secretory activity of the prostate.
- III. Of what type is the intensity of the secretory activity of the prostate gland.
- IV. Is it possible, in the experiments described above, to

make secretion-physiological observations with particular reference to the cytologic changes which ought to be present in such experiments.

V. Are the above-mentioned simple measures sufficient to cause all these variations in the working intensity of the prostatic epithelium.

MATERIAL AND METHODS.

Material: Owing to experience previously gained it was obvious that great care must be bestowed upon the selection of the test animals. In the present investigation only rabbits were used. These were all about 1 year old and weighed 2000—2250 g. The nutritive state of the rabbits was standardized as far as possible. As the animals were thus on the whole equally old and equally big, the opportunities of obtaining a similar production of the testicular hormone controlling the prostate seemed to be especially favourable. All the experiments were made early in the spring so that, in spite of all, the influence of different seasons would not be felt. In order to eliminate the possibly disturbing factors which different environment and mode of living might be thought to constitute, the males were treated by a common scheme drawn up in accordance with directions given by *Dubreuil* and *Regaud* (1909) and *Stieve* (1928), and on the basis of experiences earlier gained by the present author (1938). The rabbits were first kept in isolation for two weeks in the same room, in similar cages and on similar diet. In order to produce the greatest possible uniformity in the epithelial tissue of the prostate, the males were then placed in cages so constructed that they were separated from oestruual females, placed all around them, only by a widemeshy wire-netting. Here the males were kept for 48 hours. During this time the prostatic glands previously filled with secretion, should be brought to the desired state of repletion. Then the animals were chloroformed and the prostate portions to be examined were rapidly cut out and immediately placed in the fixingsolutions in order as far as possible to avoid any defects arising under narcosis or after death.

In this way 7 rabbits were treated. Synoptic preparations were made from 3 out of these, and 4 males were used for more detailed cytologic fixations. The whole of the urogenital system was always cut out of the animals assigned for synoptic preparations, and was placed *in toto* in the fixing-solution.

In the experiments based on copulation, 40 male rabbits in all

were used. They underwent the same preparatory treatment as that described above, and were then allowed to copulate under careful observation. In order to establish the effect a coitus interruptus without ejaculation had on the functional state of the prostate and on the intracellular structure of the epithelial cells, 4 animals were used.

Examinations after ejaculation were further made: the time elapsing from the moment of ejaculation to the moment when the animals were removed from the cages and chloroformed, was carefully determined. As described above, the glands were immediately extirpated and rapidly placed in the fixing-solution.

In this manner preparations were taken 15 sec., 3 min., 6 min., 9 min., 14 min., and 20 min. after ejaculation. For the first 4 of these 6 stages, 7 rabbits per stage were used, of which 4 were used for cytologic and 3 for synoptic preparations. For the two latter stages, only cytologic preparations of 4 rabbits per stage were made.

In this investigation in all 47 male rabbits were used.

Histological technique: For synoptic preparations, the glands were fixed in their entirety, partly in 10 per cent formol and partly in a mixture of 90 per cent alcohol and 10 per cent formol in the ratio of 9:1.

For more subtle cytologic purposes small pieces of the glands were cut out and were rapidly placed in the fixing-solutions. In this way, a number of preparations were obtained from each gland, fixed according to the same method. The following fixations were used: *Helly's Zenker-formol*, *Maximow's Zenker-formol + osmium*, *Susa-solution*, *Kopseh-Kolatsehew's*, *Altmann's*, *Kolster's solutions* and *Champy's solution* with after-chromatization according to *Benda*. Particularly in the three first-mentioned methods of fixation, preparations fixed for unequally long periods were made from each gland. The same fixing-times were then adopted throughout the whole investigation. The times adopted in the staining of the preparations according to the different methods were also carefully regulated.

All the synoptic preparations were stained according to *Weigert's* iron hematoxylin method with subsequent eosin staining. For the study of the connective tissue immediately surrounding the epithelium and of the plasma, nuclei, secretion granules and »Kittleisten« of the epithelial cells, preparations were stained which had been fixed by *Helly's Zenker-formol*, *Maximow's Zenker-formol + osmium* and *Susa-solution* according to the following methods: 1) *Bensley's* staining with brasilin + phosphoric wolfram acid and water-blue-phosphoric molybden acid; 2) *Pappenheim-Unna's* methylene-green

pyronin solution; 3) *Heidenhain's* azan method and 4) *Heidenhain's* iron hematoxylin staining.

The chondriome preparations were stained according to *Altmann-Kull* after fixation in *Altmann's*, *Champy's* and *Kolster's* solutions. It is worthy of note that the most uniform and reliable results were obtained after fixation by *Altmann's* and *Kolster's* methods.

In order to distinguish the constituents of the mitochondria from secretion granules a careful comparison was necessary between all the preparations made in this investigation.

For making visible of *Golgi's* »Apparato reticolare interno«, which for the sake of brevity will be termed the Golgi apparatus below, the *Kopsch-Kolatschew* method only was adopted. Earlier experiments have shown that if only *Bowen's* (1928 a/b) directions for this method are observed, the other methods for making visible of the Golgi apparatus are comparatively unreliable. In this investigation the Golgi apparatus was studied following an after-staining with osmium of 8—13 days. 1 per cent osmium tetroxid solution at a temperature of $+ 33^{\circ}$ C. was used. As pointed out by *Ries* (1935) and as I have myself been able to observe (1938), it is not sufficient only to make the Golgi apparatus visible. The investigator should also be in a position to compare the appearance of the Golgi apparatus during unequally prolonged periods of osmium-staining.

The dimensions of the epithelial cells and their nuclei were measured in preparations which had been fixed in formol-alcohol and stained by *Weigert's* iron hematoxylin and eosin. In order better to compare the results, only such cells were measured as lined the sides of the long epithelial palisades. It was thought important that the cells should actually be measured in the places where they were longest, respectively broadest.

For each stage, means were then valuated for these dimensions. These do not claim to constitute an accurate measure of the dimensions of the cells and nuclei in the different functional states of the prostate gland. They are only an attempt to illustrate the variations in volume of the epithelial cells and their nuclei during the different functional stages of the gland.

INVESTIGATIONS.

In this chapter an account will be given of the different preparations; each experimental stage constitutes a separate unit.

As shown in plate 1, the prostate gland lies in a caudal direction from the vesicular gland on the ventral side of the excretory ducts of the latter. With its most proximal portion, the prostate reaches a short distance below the caudal portions of the seminal gland. In a lateral direction, the prostate extends over the excretory ducts of both the seminal and vesicular glands. As also observed by *Leydolph* (1930), the prostate gland, departing from the centre, together with the vesicular gland, opens into the two distally directed urethra branches which extend towards the excretory ducts of the vesicular and prostate glands. »Die Mündung erfolgt durch 4—6 feine Ausführungsgänge, die kurz hintereinander mit je drei Öffnungen auf jeder Seite einmünden.«

The gland is of tubulo-alveolar structure. A cavity common to all glandular ducts is lacking. The epithelium reaches with numerous, often ramified folds into the lumina of the glandular ducts. In some cases, the glandular duct is furnished with narrow walls of connective tissue which on both sides are lined with glandular epithelium. Thus the original duct is divided into smaller ducts. In the proximal, medial portions of the gland, the epithelial palisades are less numerous and lower. The high prostate epithelium is also here observed to be somewhat lower and narrower. The nuclei which are otherwise round and situated basally, here become oval and lie in the centre of the cell. Thus the prostate epithelium gradually changes into the epithelium of the excretory ducts.

The prostate gland differs even macroscopically, by its opaque granular structure, from the vesicular gland with which it is intimately united.

The uniformity which according to *Schaap* (1899) and *Leydolph* (1930) characterizes the prostate gland in rabbit, is in synoptic preparations revealed by the powerful septum of connective tissue, running in the mid-line of the vertical plane, which in slightly enlarged reproductions is visible in many animals. It should be pointed out, however, that, in some cases, it was rather difficult to observe this septum of connective tissue in the prostate of the rabbit.

As pointed out by previous researchers, the size of the prostate gland is not always proportionate to the size of the animal. These experiments also showed that neither the size of the animals nor their external conditions of life were necessarily in all cases a factor regulating the size of the prostate. The prostatic epithelium, on the other hand, could be influenced by these factors. These changes were highly similar in the different animals after the same experiment.

a) NORMAL CASES AND OBSERVATIONS BEFORE EJACULATION.

(See plates 2 and 3).

The interstitial connective tissue. The connective tissue which supports the epithelium in the narrow rugae extending towards the lumina of the glandular ducts, is narrow and appears to be squeezed together. The capillaries are compressed and contain only a small number of erythrocytes. The tissue which surrounds the large ducts is also rather narrow and does not seem to contain a fair amount of liquid.

The epithelium is single-lined, cylindric. A large number of preparations reveal structures which might constitute parts of a basal membrane (see e.g. plates 3, 7, 8 and 10). Towards the lumen the cells are demarcated by a sharp, often undulatory, contour-line. The cells are also distinctly delimited towards each other.

The epithelial cells are lowest on the ridges of the epithelium palisades and highest on the sides of the palisades. On an average the cells are $25,6 \mu$ high and $6,7 \mu$ wide.

In places typical basal cells are visible (plate 4). They are, however, fairly rare. In cross section they much resemble equilateral triangles. As a rule they are fairly long. Their nuclei are large as compared to the cell. The shape of the nuclei is usually round or oval in the direction of the subjacent tissue. The basal cells are stained like the large epithelial cells by azan impregnation and stand out in marked contrast to the subjacent clear blue connective tissue.

The »Kittleisten« are visible in a large number of different preparations. They are particularly distinct after fixation in Susa solution or formol alcohol and staining with Heidenhain's iron hematoxylin or Weigert's iron hematoxylin-eosin. In this stage the »Kittleisten« are thin and clear. In cross section the cells are penta-

gonal to septagonal polygons; the hexagonal type seems to be most common, however.

The nucleus. The cells are uninucleated. In this stage the typical position of the nucleus is in the most basal part of the cell so that only a narrow plasma layer separates the nucleus from the basal wall of the cell. However, the nuclei in some cells may be observed closer to the centre of the cell.

As a rule the nuclei are round or slightly flattened out towards the base of the cell. If the cells are closely packed together, this may also affect the shape of the nucleus so that the nucleus becomes squeezed together from the sides.

The nuclei are usually $5.5\ \mu$ high and $6.4\ \mu$ wide. In the different cells the nuclei seem to have about equally large volumes.

The nuclei are in all cells well circumscribed. Not a single preparation made for the present investigation revealed mitoses or other signs of cell-division.

In the nuclei 2-3, rarely 4, nucleoli are visible. The chromatin forms a finely meshed network which consists of thin distinctly contoured chromatin fragments and small, bright vacuoles in between. The nuclei are successfully stained by the usual methods of nucleus-staining.

The plasma. The structure of the cells is clearly bipolar. The plasma *per se*, however, shows no signs of different structure in the different parts of the cell. In this connection it ought to be pointed out that by plasma we here mean only the plasma proper, all differentiated structures like mitochondria, Golgi bodies and intracellular secretion granules being disregarded.

The main part of the plasma is in this stage displaced to the small space below and lateral of the cell nucleus. In some cases, a narrow plasma margin may also be observed along the cell-walls higher up in the cell, but as a rule only an accumulation of intracellular secretion granules are here visible. The plasma in the basal parts of the cell seems to be fairly homogeneous. In preparations stained with azan or brasilin-water-blue, small drops or vacuoles are often visible at the base of the cell. The plasma is stained an even red with *Weigert's* iron hematoxylin-eosin. No pigment-resembling granules were observed in these preparations.

The chondriome. In the most basal portion of the cell, a slight accumulation of chondriome constituents are often visible, which appear to have a net-resembling arrangement. From these formations the long, upstretching mitochondria depart. Often this accumulation is so inconsiderable that it seems as if the mitochondria would depart directly from the immediate neighbourhood of the basal wall of the cell. In this stage the mitochondria are very long.

They extend upwards past the nucleus and into the Golgi zone. Typical mitochondria, however, may also be seen between the secretion drops in fairly apical parts of the cell (plate 2). Shorter mitochondria arranged in a row often turn out to be connected when the micrometer screw is turned.

The path of the mitochondria is fairly tortuous. Perhaps, in some cases, this is why the mitochondria seem to have swellings interchanging with clear indentations. In many cases, however, it is obvious that the drop-like swellings are not a result of this. Sometimes the mitochondria are seen to end in the Golgi zone just in such a small swelling. It seems as if the mitochondria might be ramified. At times, this impression is created only because two independent mitochondria are laid crosswise.

In preparations fixed in *Altmann's* or *Kolster's* solutions, the mitochondria strongly resemble each other. *Champy*-preparations often reveal signs of shrinking of the cells, the mitochondria may be shorter and more bulky, sometimes ring-shaped. Owing to these facts, the mitochondria are here described chiefly on the basis of *Altmann* and *Kolster* preparations.

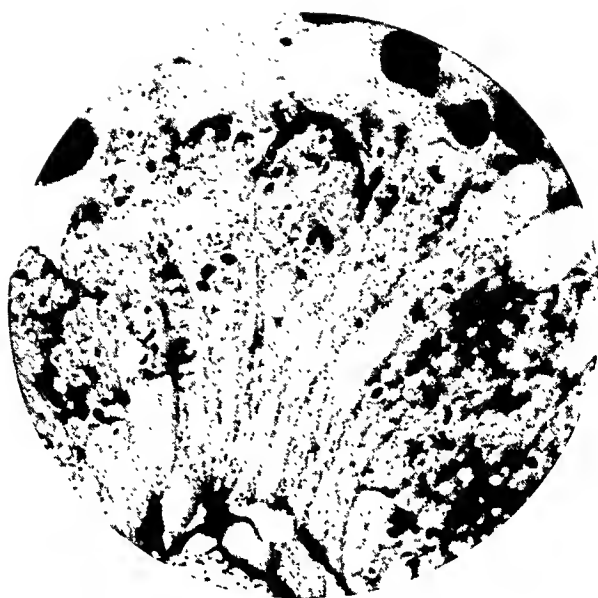
The *Golgi apparatus* lies immediately above the nucleus. In some cases, a thin plasma layer may be observed between the nucleus and the Golgi apparatus. The latter occupies about $\frac{1}{4}$ — $\frac{1}{5}$ of the space in the longitudinal direction of the cell. From the lateral walls of the cell it is separated by a fairly wide plasma layer.

In most cases the Golgi apparatus appears to be a fairly closed formation, as in plate 3. The Golgi apparatus is built up of slender rods blackened by osmium which together form a network-resembling formation. In the light meshes of this net, secretion drops of various sizes are visible. They have the same appearance as the secretion drops in the granule zone of the cell. In the black rods a small number of unequally large, faintly stained, or non-stained, vesicles may also be observed.

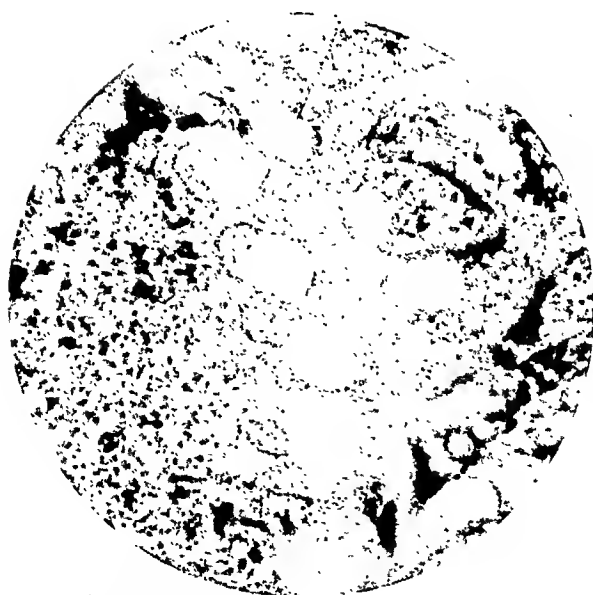
In this stage, when the cells are filled to the breaking-point with secretion granules, it may be observed that the Golgi apparatus is also so filled with drops of secretion that the rods have become more and more thinned off between the secretion granules. The osmium-stained rods then extend over a somewhat larger cell area. Moreover, they are much thinner: only slender black streaks are visible between the different drops of secretion.

Both in transverse and longitudinal sections the Golgi apparatus is seen to be somewhat lighter in its most central portion.

In chondriome preparations small lighter areas in the plasma are visible in the Golgi zone. These Golgi negatives, however, are rather indistinct in this stage. The mitochondria extend into this



*Plate 2. Stage of secretory inactivity.
Altmann, Altmann-Kull. Enlarged 1320 X.*



*Plate 3. Stage of secretory inactivity.
Kopsch-Kolatschew. Enlarged 1320 X.*

region. Sometimes the mitochondria may be seen to end in a small drop-like formation. In chondriome preparations, secretion granules of all sizes are seen in this zone crowded together just in the Golgi negative.

The intracellular secretion granules. Besides in the Golgi zone, secretion granules are present in almost the whole of the remaining part of the cell. Even infranuclear and lateral of the nucleus isolated granules may be seen. Particularly above the nucleus which as a rule is pressed against the base of the cell, secretion granules are almost exclusively present. No interspace between the granules in the Golgi apparatus and the granules found in the more apical parts of the cell can be observed. The cells are excessively filled with secretion granules so that the apical wall of the cell mostly projects into the lumen like a cupola.

The granules are stained red with eosin and yellow by *Heidenhain's* azan staining method. The granules lying closer to the centre of the cell consist of an intensely stained kernel surrounded by a light unstainable area. In the apical parts of the cell, however, the densely stained kernels are clustered together more closely and the lighter areas seem to have disappeared almost altogether.

Close to the basal wall of the cell, small light vacuoles are visible in *Kopsch-Kolatschew* preparations; such vacuoles may also be seen in a large number of other preparations. It may be asked whether these are drops of secretion of a different type from the ones described above. They might also be thought to be artefacts or structures in the plasma determined by other factors. It is possible that such vacuoles may be observed on the outer side of the basal wall of the cell. The question arises as to whether these vacuoles may be an indication of the much debated internal secretion of the prostate gland.

The extrusion. In this stage the cell is demarcated towards the lumen by a distinct and sharp contour. An expulsion of secretion granules into the lumen cannot with certainty be observed. The extrusion therefore should be very slight.

The contents of the lumen. The lumen is heavily filled. The contents consist, in a fresh condition, of a milky, fluid, finely granulated secretion. The secretion has a marked tendency to dissolve from the preparations. For the most part it consists of a faintly eosinophile, shapeless substance. In the latter are found fairly large, perhaps somewhat more intensely eosinophile drops which in *Altmann-Kull* preparations are stained a vivid red and finely granulated in distinction to the yellowish formless secretion. Further, a small number of pyknotic nuclei are visible.

In some glands the typical prostatic calculi are observed.

The general impression of the investigations in this stage is that the epithelium is in a certain state of inactivity. This state might also be interpreted as a staple stage, since the epithelial cells are filled to the breaking-point with previously formed secretion granules. As will appear from the following exposition, the term »secretion preparedness« would perhaps be most adequate in describing the state of the prostate epithelium in this stage.

The preparations made after a *coitus interruptus* without ejaculation on the whole reveal the same state of the prostatic epithelium as in the stage described above. The most obvious difference no doubt is found in the connective tissue surrounding the glandular ducts which is now subject to a certain hyperemia. — That the epithelium in this stage should still be inactive is natural, since this condition was determined by the pressure exerted on the abundant contents by the walls of the gland. Prior to the ejaculation this pressure is still unaltered.

b) PREPARATIONS TAKEN 15 SECONDS AFTER EJACULATION.

(See plates 4 and 5).

Shortly after the lumen has been emptied of its contents through ejaculation, the prostate gland reveals obvious changes.

The interstitial connective tissue. The increased contents of blood in the small arteries of the surrounding connective tissue has spread as far as the smallest capillaries of the epithelial lobes. Numerous erythrocytes are visible in the somewhat wider and more porous connective tissue in the epithelial folds. It seems as if its content of liquid had also increased somewhat.

The epithelium. The formerly distended glandular ducts have noticeably shrunk after the secretion has been emptied. The epithelial folds turned towards the lumen are perhaps somewhat longer, but they do not seem to have increased in number. The epithelium has not become higher. The cells are now on an average 25μ high and 7μ wide. Towards the connective tissue the epithelium is still demarcated by the earlier described contour-line resembling a basal membrane.

The »Kittleisten« are thin and distinct as before.

The nucleus. The nuclei still lie very close to the basal wall of the cell. They are mostly of a round shape. Their diameter in the longitudinal direction of the cell is 7μ and their transverse diameter is 8.8μ . Towards the plasma the nuclei are well demarcated in the preparations by a clear and unbroken nuclear membrane. The

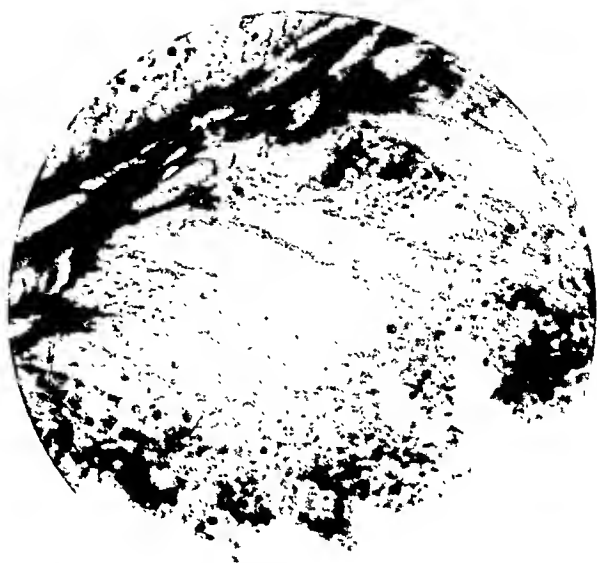


Plate 4. Beginning strong secretion. *Allmann.*
Allmann-Kull. Enlarged 1320 \times .

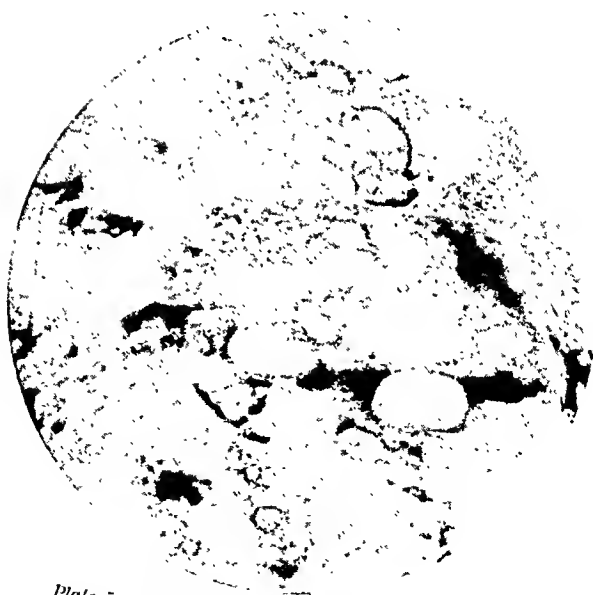


Plate 5. Beginning strong secretion. *Kopsch-*
Kolatschew. Enlarged 1320 \times .

nucleoli have not changed. The chromatin still forms a fine-meshed net which much resembles the one found in the former stage.

The plasma in this stage begins to assume a clear basophile character in the most basal portions of the cell. The plasma has perhaps assumed a somewhat more foamy appearance.

The chondriome. Close to the basal wall of the cell, slight or no accumulations of chondriome material are seen. The number of the mitochondria does not seem to have been altered. As a rule they are situated in the longitudinal direction of the cell. The long mitochondria which were typical of the previous stage are no longer so frequently observed. The mitochondria are now as a rule somewhat shorter. Their contours are uneven on account of the small droplike swellings which frequently occur in this stage. No structural differences were observable in the mitochondria. They extend towards the Golgi zone in which they are often observed to terminate in a small drop-like formation. It is difficult to find mitochondria between the secretion drops in the most apical portions of the cell.

The Golgi apparatus is situated just above the nucleus. In some cases, the most basal part of the Golgi apparatus is seen to surround the apical portion of the nucleus like a mantle. The volume of the Golgi apparatus has increased in the longitudinal direction of the cell. Transversally, it is separated from the wall of the cell only by a narrow plasma zone. In many cells, the osmium-stained net may reveal a very complicated form. As a rule the Golgi apparatus is open as it were towards the apical part of the cell and closed towards the base of the cell. In many cases, however, the Golgi apparatus is also open towards the base of the cell.

The osmium-stained net is of a very porous appearance. The interspaces between the osmium-stained rods consist of smaller and larger vacuoles. The vacuoles, in a somewhat irregular manner, seem to be lighter than the plasma on the external side of the Golgi apparatus. The osmium-stained rods are fairly wide. They are irregular and nodose. In addition to these small black nodules, small light vesicles of different size are seen in the Golgi apparatus. The vesicles are surrounded by the substance blackened by osmium. In places, this black substance has sometimes thinned off or wholly disappeared so that the light drop freely communicates with its surroundings. In the light meshes of the Golgi net, drops are seen having the same appearance as the secretion drops in the apical part of the cell.

In *Altmann-Kull* preparations, light areas are observable in the Golgi zone. This Golgi negative reveals secretion granules of varying size from exceedingly small drops to fully developed drops. In

synoptic preparations, the Golgi negatives appear very indistinct.

The intracellular secretion granules. Isolated secretion granules are still visible basal of the Golgi apparatus. As a rule, the most basal granules are observed just in the Golgi apparatus. Granules of different sizes are present in the latter, from quite small up to equally large with those situated apically of the Golgi apparatus. Typical of the granules lying in the neighbourhood of the Golgi zone is the large light area surrounding the darker and easily stainable, central, grain-resembling formation. The more apical the secretion granules are situated in the cell, the narrower and more indistinct is the light area. Close to the lumen it seems as if the drops of secretion consist solely of the central grains. They are also here more frequent and appear as it were packed together.

From the Golgi zone to the most apical part of the cell, a continuous mass of granules is visible. No confluence of the separate granules into uniform large lumps is noticed. The central grains in the secretion drops, however, are more sharply stained the closer they lie to the Golgi zone. Azan staining of *Maximow-osmium* preparations yields particularly fine and clear preparations from this point of view.

The extrusion. Obvious signs of an increased extrusion are noticeable. In hematoxylin-eosin preparations it is still possible to observe a comparatively complete and distinct wall towards the lumen. To the side of the cell wall which is turned towards the lumen, however, are attached a large number of secretion drops which have evidently just been expelled or are in the process of being expelled from the cell. Particularly in the chondriome and Golgi preparations, the apical cell wall, on the other hand, is in places observed to be considerably more indistinct. As shown in plates 4 and 5, only fragments of the cell wall are seen. Through the gaps which have been formed the secretion granules of the cell flow out into the lumen. The preparations do not distinctly show how secretion granules pass the wall of the cell. In *Altmann-Kull* preparations, for instance, it is sometimes possible to observe how the small red granules have been extruded a short distance into the lumen without losing their individuality. As a rule, however, it seems as if the extruded drops would flow together fairly rapidly, losing the stainability typical of the intracellular secretion granules.

The contents of the lumen seem to be very scanty. Some faintly acidophile, or neutrophile, shapeless secretions are observable, however. A number of larger drops are also seen which have the same staining affinity, even if they appear to consist of a somewhat more compact substance. A small number of prostatic calculi are seen in some glands.

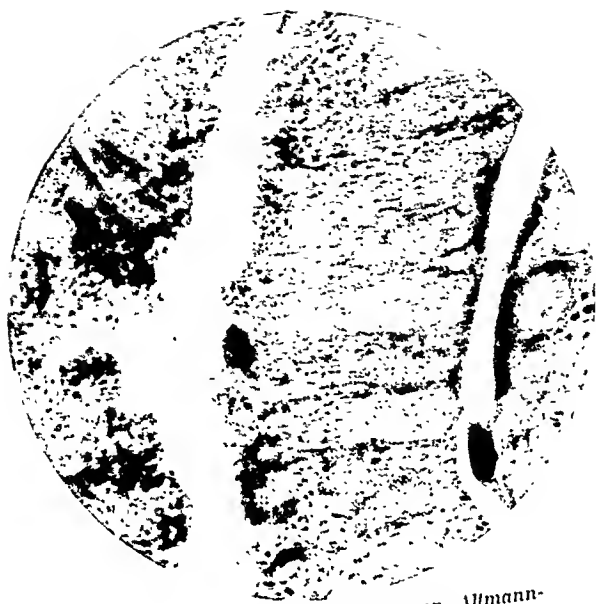


Plate 6. Strong secretion. Altmann. Altmann-Kull. Enlarged 1320 X.



Plate 7. Strong secretion. Kopsch-Kolatschew. Enlarged 1320 X.

c) PREPARATIONS TAKEN 3 MINUTES AFTER EJACULATION.

(See plates 6 and 7).

The *interstitial connective tissue* contains a somewhat smaller number of erythrocytes than in the preceding stage. In the exterior parts of the epithelial folds, only isolated red blood corpuscles are observable. The connective tissue nearest the epithelium, on the other hand, contains a fairly large amount of liquid: the connective tissue is stained a lighter colour and has a more porous appearance than before.

The *epithelium*. The glandular ducts have on the whole the same appearance as in the preceding stage. They do not seem to be distended by the contents of the lumen. Neither do the glandular lumina appear to be enlarged. The epithelium is demarcated towards the connective tissue by a thin basal membrane. The cells are now on an average $29,8\mu$ high and $7,7\mu$ wide.

The »*Kittleisten*« are as before rather thin but distinct.

The *nucleus*. The nuclei still lie in a markedly basal direction, but the distance to the basal wall of the cell is perhaps just a trifle longer. The shape of the nuclei is unaltered. On an average, they are $7,3\mu$ in the longitudinal direction of the cell and $6,7\mu$ in the transversal direction. They are separated from the other contents of the cell by an unbroken and clear nuclear membrane. The nucleoli have not undergone noticeable changes. The border-line between the chromatin rods and the light vacuoles seems to be somewhat diffuse. These vacuoles appear to have become lighter than in the preceding stage.

The basophiles of the *plasma* have in this stage spread over the largest portion of the intranuclear cell region and also extend some distance in an upward direction between the nucleus and the lateral walls of the cell. The plasma is fairly foamy.

The *chondriome*. In this stage, also, it is possible to observe small conglomerations of chondriome substance in many cells nearest to the basal cell-wall. The mitochondria are more numerous than before. As a rule they are comparatively short and thick. The nodose structure from the preceding stage is no longer as clearly visible. The mitochondria extend in the longitudinal direction of the cell and their path is remarkably straight. One gets the impression that a distinct adjustment on the part of the chondriome has taken place from the base of the cell in the direction of the Golgi zone. Here also, the mitochondria are frequently seen to terminate in a small drop in the Golgi zone. Above the Golgi zone no mitochondria are as a rule observed.

The *Golgi apparatus* has no longer the same volume as before.

It has become smaller both in the longitudinal and transversal directions. The osmium-blackened net no longer encloses part of the nucleus. In most cases a free zone may be clearly observed between the Golgi apparatus, which now has a more apical position, and the nucleus. But the Golgi apparatus rarely has a more apical position than in the central portions of the cell.

In particular it is the internal structure of the Golgi apparatus that reveals changes in this stage. We may observe that the osmium-stained rods have become somewhat reduced in number and in part somewhat thinner. Relatively speaking, the light contents of the apparatus are now predominant. Numerous secretion granules are found in the meshes of the osmium-stained net. These granules have a typically darker centre surrounded by a light area. These drops seem to lie in a somewhat darker, surrounding medium which occupies the remaining large meshes of the net. In the osmium-stained rods themselves, a large number of light vesicles of varying size are visible in this stage, too. The osmium-blackened walls which surround these light drops, may also be seen to thin off and are in some places wholly lacking so that the drop is free to emerge into the surrounding cellular space.

In *Altmann-Kull* preparations, the Golgi negatives are fairly distinct in this stage. In synoptic preparations stained e.g. with hematoxylin-eosin, the Golgi negatives, on the other hand, appear very indistinctly.

The intracellular secretion granules. Such typical secretion granules as are seen in the apical parts of the cell are now rarely observed in cell portions lying basally of the Golgi apparatus. In the latter the secretion granules have the same appearance as in the preceding stage. Secretion granules are continuously present from the Golgi zone as far as the most apical portions of the cell. One gets the impression that the granules are no longer packed as closely together as formerly. The light area which is so typical of the basal secretion granules is now also better retained by such granules as have a more apical position in the cell.

The extrusion. In this stage the apical cell-wall is most indistinct. Often it is difficult to observe at all any clear cell-wall. The extrusion seems to be very active to judge from the amounts of secretion emerging from the cells. No observations were made indicating that large portions of the cells are detached in one operation. It is only the secretion granules found in the cells that are extruded. In proportion as these granules are excreted into the lumen they gradually lose their individuality, flowing together into larger drops which form the contents typical of the lumen.

The contents of the lumen. The lumen has become somewhat more filled. The composition of the contents is as before.

d) PREPARATIONS TAKEN 6 MINUTES AFTER EJACULATION.

(See plates 8 and 9).

The interstitial connective tissue contains a fairly small number of erythrocytes, but the content of liquid seems to about the same as in the preceding stage. The small blood vessels which are found somewhat more exteriorly in the surrounding tissue, are still heavily filled with red blood cells.

The cpithelium. The glandular lumina seem to be more filled with secretion than before. So far, however, it cannot be observed that the contents of the lumen distend the glandular ducts to any marked extent. In this stage, too, a basal membrane, not very pronounced in places, was observed. On an average, the epithelial cells are $28,5\ \mu$ high and $7,3\ \mu$ wide.

The »Kittleisten« are distinct and seem to be slightly wider.

The nucleus. In this stage the nuclei are in their most apical position. This, however, does not even result in their lying as high as the middle of the cell. But a fairly broad plasma zone is seen between the basal cell-wall and the nucleus. The shape of the nuclei still approaches the spherical form. Their average dimensions are $7,2\ \mu$ in the longitudinal direction of the cell and $6,2\ \mu$ in the transverse direction. They are surrounded by a clear and unbroken nuclear membrane. The number of the nucleoli are as before. Their stainability in azan preparations may have increased somewhat. The meshes in the chromatin net now appear to be fairly large and light. The borderline between these meshes and the chromatin fragments is not as sharp as earlier.

The basophiles of the *plasma* in this stage attain their largest extension. It reaches from the base of the cell past the nucleus a fair distance towards the more apical parts of the cell. The plasma is now clearly foamy.

The chondriome. In this stage the accumulations of chondriome material have disappeared at the basal walls of the cells. The mitochondria are also fairly few in number. Their path is straight, not tortuous, in the longitudinal direction of the cell. They are very thin and have an even outline. The small drop-like swellings are seen only rarely except in the Golgi zone. The mitochondria are stained comparatively faintly in this stage. The mitochondria often reveal a tendency to break up into bead-like formations arranged in a row. We get the general impression that a certain shortage of material manifests itself in the chondriome.

The Golgi apparatus. The osmium-stained net is still situated approximately in the middle of the cell or in some cases in a more apical direction. The osmium-blackened rods have become increas-

ingly fewer and thinner. They take up a fairly large space in the cell. In the rods are still visible a number of small light vesicles of the same appearance as previously described. In the wide meshes of the osmium-stained net a large number of secretion granules are observed. As described above, these are made up of a somewhat darker centre surrounded by a clear area. The meshes of the Golgi net thus assume a somewhat foamy and very light appearance. — A fairly large area which exclusively shows such a light and foamy composition, is now frequently observed, particularly just basally of the thin osmium-blackened rods. This region evidently belongs to the Golgi apparatus. It seems possible that the dark rods have here become stretched out between the drops of secretion to such a degree that they are almost invisible.

In chondriome preparations a fairly clear negative copy of the Golgi apparatus is seen. Between a large number of granules of varying size are seen light, lamella-resembling formations which occur irregularly between the granules and which also in part surround the accumulations of granules in the Golgi zone. In the synoptic preparations the Golgi negatives are clearest in this stage.

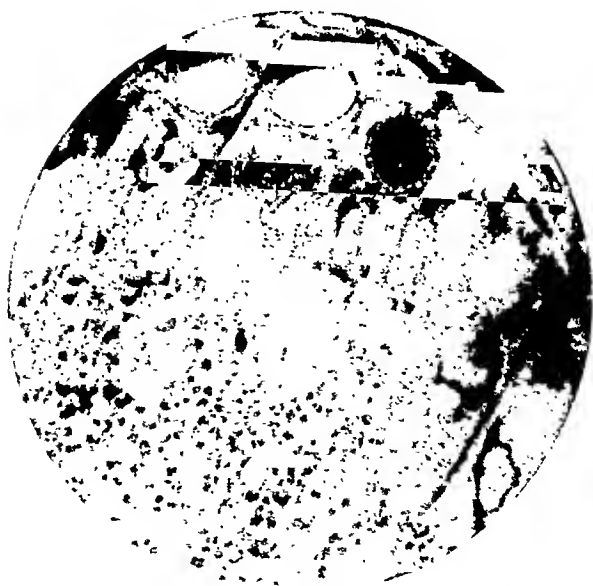
The intracellular secretion granules. In this stage, too, only isolated secretion granules are seen basally of the Golgi zone. Secretion granules which earlier occupied the larger portion of the cell are now only seen as a comparatively small conglomeration in the apical half of the cell. In some cases, the secretion granules in the cell may be very few in number, in other cases they are more frequent above the Golgi zone. In the Golgi zone itself, secretion granules of varying size are also observed. In the more apical parts of the cell, granules in this stage have fairly well preserved light areas which surround the central, well stainable grains. Is this possibly due to the fact that the intracellular secretion granules are now comparatively few in number?

The extrusion no longer appears to be of the same intensity as before. The apical wall of the cell shows fairly clearly in most cells. No gaps are as a rule observable in this cell-wall. On the side turned towards the lumen, however, are seen numerous adherent drops of secretion, indicating a rather active extrusion.

The contents of the lumen. In the lumen a fairly abundant secretion is observed. It consists mostly of a shapeless substance in which are scattered small drops which may exhibit a rather sharp contour. The stainability of the secretion is as before.



*Plate 10. Fading secretion. Altmann.
Altmann-Kull. Enlarged 1320 X.*



*Plate 11. Fading secretion. Kopsch-
Kolatschew. Enlarged 1320 X.*

e) PREPARATIONS TAKEN 9 MINUTES AFTER EJACULATION.

(See plates 10 and 11).

The interstitial connective tissue contains a very small number of erythrocytes. The abundance of liquid previously observed has decreased considerably. The connective tissue markedly resembles the stage before copulation.

The epithelium. The glandular ducts now seem to be fairly well filled and one gets the impression that the walls already begin to distend, although to a rather small degree, by the secretion produced. In this stage, too, a thin basal membrane may be observed. The cells are now on an average $28,6 \mu$ high and $7,3 \mu$ wide.

The »Kittleisten« are clear. In some places a splitting may be observed to the effect that each cell gets a darker lamella and between the latter a lighter fissure is visible. Intercellular bridges extending across the light interspace could not be observed in these preparations. In transverse sections, the »Kittleisten« form a net which is thickest in the corner-points.

The nucleus. The nuclei are again pressed lower down towards the base of the cell. Their shape is not altered. They are on an average $7,4 \mu$ in the longitudinal direction and 6μ in the transverse direction of the cell. They are surrounded by a clear nuclear membrane. The nucleoli are unaltered. Their stainability seems to have decreased somewhat. The chromatin net has assumed somewhat more distinct contours. The meshes in the chromatin net have also become a little smaller and possibly somewhat darker.

The plasma. The basophile character of the plasma has now been considerably reduced. Mainly in the internuclear cell regions it is still possible to observe a basophilic staining of the plasma. Neither does the foamy appearance show as clearly as in the preceding stage.

The chondriome. Accumulations of chondriome material at the basal cell-walls are present in many cells. As a rule, however, they are fairly inconsiderable. The mitochondria are comparatively few in number. They seem to constitute a direct continuation of the basal chondriome formations. The path of the mitochondria is somewhat more tortuous than before. Their contour is even as a rule, but a small number of drop-like swellings may be observed. The mitochondria stain well with oxygen-fuchsin. They still extend towards the Golgi zone where they are often seen to end in small droplike formations. In this stage mitochondria are again easily observable above the Golgi zone in the most apical parts of the cell.

The Golgi apparatus lies immediately above the nucleus. From

the latter it extends in some cases even fairly high up in the cell. Its volume, however, has decreased in some measure.

The rods blackened by osmium have become somewhat thicker. They are no longer seen to contain the abundance of small light vesicles that were visible before. The light meshes in the Golgi net have become smaller but possibly somewhat more numerous. In the meshes distinct secretion granules are seen. Golgi bodies are present which are highly reminiscent of the stage before copulation. As a rule it may be said that the Golgi apparatus in this stage has become more closed in character.

In *Altmann-Kull* preparations distinct Golgi negatives are observed but they are no longer as clearly outlined as in the preceding stage. In synoptic preparations, also, indistinct lighter areas are still observable in the Golgi zone.

The intracellular secretion granules. In this stage, too, extremely few secretion granules are visible basally of the Golgi zone. Otherwise, the apical portion of the cell is filled with secretion granules which appear in unbroken succession from the apical end of the cell to the most apical limit of the Golgi zone. Thus, secretion granules again occupy the larger portion of the cellular space. They seem, however, not to be so packed together as before copulation, neither do they as yet occupy an equally large portion of the cell.

Secretion granules lying closer to the apical end of the cell mostly become darker in osmium than the granules which lie in or nearer the Golgi zone. On the other hand, the secretion granules lying more basally in the cell are seen to be more densely stained than those which lie in a more apical direction. Granules lying nearer the Golgi apparatus are frequently stained a more distinct red with azan than the bright yellow secretion granules lying in a more apical direction. Possibly, this may serve as an indicator of the further development of the secretion granules within the cell.

The extrusion appears to have decreased still further in intensity. A rather sharp and uniform cellular wall towards the lumen may be observed in most cases. Judging from the unequally large secretion granules adherent to the side turned towards the lumen, a fairly active extrusion seems to be going on.

The contents of the lumen. The lumen contains an abundance of secretion of the same composition as before. In some glands prostatic calculi are observed.

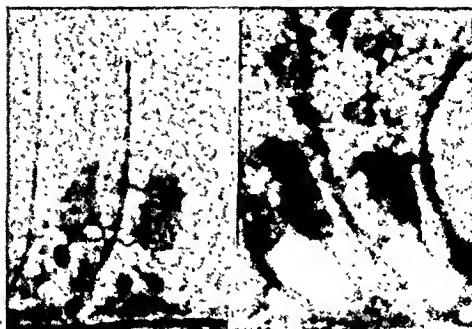


Plate 12. Comparison between a Golgi apparatus
 on a *Champy, Altmann-Kull* preparation (to left)
 and on a *Kopsch-Kolatschew* preparation (to right).
 Notice the small secretion granules
 in Golgi apparatus.

f) PREPARATIONS TAKEN 14 AND 20 MINUTES AFTER EJACULATION.

(See plate 12).

As the preparations referring to these two experimental stages do not markedly deviate from one another, they are here described together.

The return to the stage before copulation seems to have continued to a fairly marked degree. In the *interstices of the connective tissue*, only a slight blood-supply is seen. The *epithelium* and the »Kittleisten« seem to have the same appearance as before copulation. The same applies to the *nuclei* and *plasma* of the epithelial cells. The *chondriome* also presents marked resemblances. Nearest the basal wall of the cell, fairly slight accumulations of chondriome material are frequently seen. The mitochondria have gradually become somewhat more numerous. From one stage to another they also become somewhat longer. They have a slightly sinuous course and reach from the base of the cell to the Golgi apparatus. The contour of the mitochondria is fairly even. The position and appearance of the *Golgi apparatus* are highly reminiscent of conditions before copulation. The fragments resemble those to be seen before copulation, even if the meshes in the osmium-stained net are smaller. In the latter, secretion granules of varying size are still observable. The *intracellular secretion granules* are numerous. They occupy the cellular space from the most basal part of the Golgi zone to the apical wall of the cell. As yet, however, they have not become so crowded that some secretion granules are pressed down below the cell-nucleus. The *extrusion* still seems to be comparatively active in the former stage. 20 minutes after copulation, however, it looks as if the secretion had diminished still further. The extrusion is now very slight. The *contents of the lumen* have increased. Its composition is the same as formerly.

It consequently seems as if the production of secretion in the prostate gland would gradually decrease. Eventually the exclusion becomes quite inconsiderable and probably remains like that until the lumen of the gland is again emptied of its secretion.

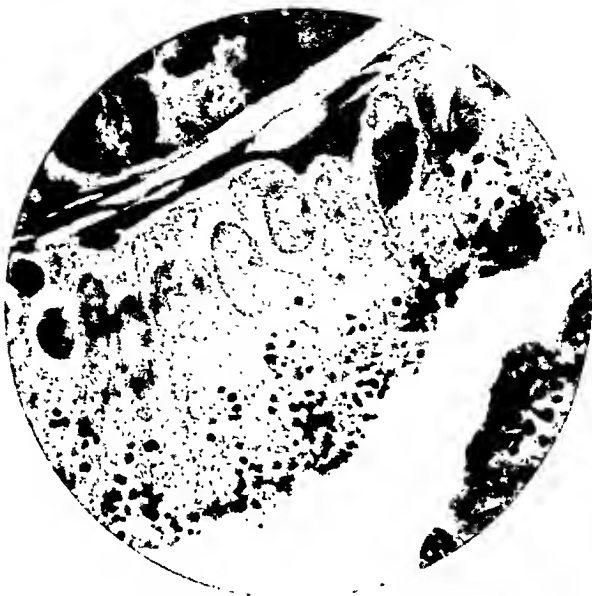
DISCUSSION.

1. *The interstitial connective tissue.* In the prostate gland, heavily filled with secretion in normal unmated rabbits, the

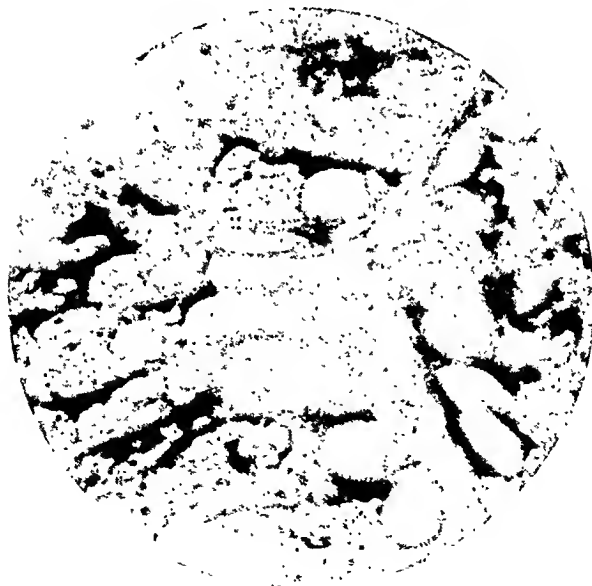
connective tissue which immediately surrounds the different glandular ducts appears to be fairly compressed and comparatively poor in liquid. The same impression is created by the connective tissue supporting the narrow epithelial lists which project into the lumina of the glandular ducts. The largest portion of the capillaries of the prostate are, according to *Stieve* (1930), found in the connective tissue nearest the glandular ducts. The capillaries reach out into the epithelial lists where they form fine loops. As a rule, only a few number of erythrocytes are seen in the latter. Evidently, the interstitial connective tissue is the site of a secondary anemia. On sexual stimulation, signs of a certain hyperemia may be seen in the small blood vessels which are found somewhat farther away from the glandular ducts.

Soon after the prostate gland has been emptied of its contents through ejaculation and the pressure of the secretion on the distended walls of the gland has become eliminated, the connective tissue everywhere becomes broader and assumes a more porous appearance. The hyperemia in the somewhat larger blood vessels which was present even prior to ejaculation after strong sexual stimulation, now spreads as far as the thinnest capillaries in the connective tissue of the epithelial lists. A general increase in the content of liquid is also noticeable.

As soon as 3 minutes after ejaculation this hyperemia is seen to begin to decrease. The content of liquid, on the other hand, is very high in the connective tissue which owing to this becomes more mellow and porous. The content of liquid is then kept constant for a while but gradually it begins to decrease. The number of erythrocytes in the connective tissue and its content of liquid then decrease along parallel lines so that the connective tissue as soon as 9 minutes after ejaculation is fairly suggestive of the connective tissue before copulation. This resemblance becomes clearer still the longer is the interval that is allowed to pass after ejaculation. The more the lumina of the glandular ducts are again filled with secretion, the more do the walls of the glandular ducts distend



*Plate 8. Diminishing, but still rather strong
secretion. Altmann. Altmann-Kull.
Enlarged 1320 X.*



*Plate 9. Diminishing, but still rather strong
secretion. Kopsch-Kolatschew.
Enlarged 1320 X.*

and the connective tissue assumes an appearance which is more compact and poorer in liquid.

2. *The epithelium* is high-cylindric. In the heavily filled glands before ejaculation, the epithelium, however, is somewhat lower than in the stage where the lumina of the glandular ducts have been emptied of their contents. When the secretory activity is most pronounced, the epithelium is at its highest, then it gradually again becomes somewhat lower.

Towards the connective tissue the epithelium is demarcated by a very thin basal membrane which in some cases may be rather difficult to observe. Regarding the occurrence of this basal membrane different opinions have been expressed. In the rabbit it was observed by *Stilling* (1884), in the dog by *Walker* (1899), in man by *Eberth* (1904), and finally, in the rat by *Moore, Price and Gallagher* (1930). *Oppel* (1904) and *Leydolph* (1930) refuse to admit the presence of a basal membrane in the prostatic epithelium of the rabbit. According to *Langerhans* (1874), *Weski* (1903) and *Schaffer* (1922), the prostatic epithelium in man also lacks a basal membrane.

In the prostatic epithelium of the rabbit, basal cells are fairly scarce. They are small and inconsiderable. Only a narrow plasma zone surrounds the nucleus which is comparatively large for the dimensions of the cell. *Stilling* (1884), *Schaap* (1899) and *Petersen* (1909) also described these basal cells, but *Leydolph* (1930) was unable to observe them.

As it is sometimes possible to observe in the epithelium cells which, judging by appearances, are dead, and as a number of pyknotic nuclei may also be found in the lumina of the glandular ducts, we have to conceive of the possibility that new cells may also be formed in the prostatic epithelium. As, however, in the preparations made in the present investigation, mitoses were in no case observed, it seems reasonable to assume that the small basal cells themselves in some way give rise to the new epithelial cells.

According to *Rauther* (1904), the free epithelial margin towards the lumen was in most cases indistinct in the pro-

static epithelium of the rabbit. *Stieve* (1930) also found in man that the thin membrane towards the lumen could sometimes be broken through. It was shown in the present investigation that the clear undulatory line of demarcation towards the lumen, described by *Leydolph* (1930), was particularly distinct in the secretorily inactive experimental stages. In a state of intense secretion this line could be almost wholly obliterated.

3. *The »Kittleisten«*. In the prostate gland in rabbit the »Kittleisten« form a clear net. In the prostatic epithelium in man a similar net was described by *Stieve* (1930). The preparations show that this net is thickest in the corner-points. During, or perhaps particularly soon after secretory activity, the »Kittleisten« are seen to become somewhat thicker. According to *Wahlberg* (1933), the thickness of the »Kittleisten« in the thyroid epithelium is directly proportional to the cellular activity. In active stages, the »Kittleisten« easily split so that each cell gets its portion. This might possibly be interpreted so that each cell contributes to the construction of the »Kittleisten« with one half. Between these split walls a lighter fissure is usually observable. It was not possible, by the methods here employed, to observe in these fissures such thin bridges as, according to *Chlopakow* (1928), unite the contents of adjacent cells.

If the section was cut obliquely to the longitudinal direction of the epithelial cells, the »Kittleisten« in the prostatic epithelium are seen to resemble such strokes and points as were described in other glandular epithelia by *Schaffer* (1927) and *Wahlberg* (1933).

4. *The nucleus*. The cells are uninucleated. The nucleus is as a rule found in the most basal part of the cell. In a stage of intense secretory activity, the nucleus is observed to have a more apical position. It may be found in the middle-most portions of the cell, but rarely in a still more apical position. That the nucleus in this way becomes displaced during secretion was also observed by *Stilling* (1884), *de Bonis* (1907) and *Petersen* (1909). It seems as if the displacement

of the nucleus occurs in a completely passive manner and is due partly to the fact that the numerous grains of secretion which earlier filled the cell and pressed the nucleus towards the base of the cell, have now largely been excreted from the cell, and partly to the fact that the nucleus is caught in the general flow of cellular contents or secretory material from the base of the cell towards the lumen. When the extrusion is no longer equally intense, and when the largest portion of the cell again begins to be occupied by secretion granules, the nucleus is again pressed down towards the base of the cell.

The shape of the nuclei is not subjected to great modifications. In cells where the extrusion is one of minimum intensity and the largest portion of the contents of the cell is made up of secretion granules closely packed together, the nucleus is seen to be somewhat flattened out towards the base of the cell. In a state of intense secretion, a slight increase in the volume of the nucleus may be observed.

In the preparations, the cells were always distinctly demarcated by a clear nuclear membrane. The latter was also described by *Walker* (1899), *Stieve* (1930) and *Macklin* and *Macklin* (1932). In none of the experimental stages in the present investigation did the nuclear membrane seem to be broken or otherwise changed, so that it would have rendered possible the emergence of visible nuclear constituents into the plasma.

The number of the nucleoli was not modified in the different experiments. However, their stainability appeared to be somewhat increased, e. g. by *Heidenhain's* iron hematoxylin and azan method, when the cell was in a state of intense secretion. *Stilling* (1884) also pointed out the distinct nucleoli after copulation.

The chromatin is usually made up of thin, distinctly outlined fragments which together form a thin, fine-meshed network with numerous small vacuoles of a lighter colour. According to *Walker* (1899) the chromatin is clearly outlined at the beginning of secretion. In the present investigation it

was shown that the borderline between the chromatin rods and the meshes in the chromatin net had become somewhat diffuse as soon as 3 minutes after ejaculation. Somewhat later, the border-line between the now fairly porous chromatin fragments and the comparatively large light vacuoles is still seen to be rather indistinct. When the secretory intensity of the cell begins to decrease, the chromatin fragments again become more distinctly marked and the vacuoles become smaller. It seems as if these changes in the nucleus were at least in part determined by an increased content of liquid in states of increased secretory activity in the cell. Possibly, the slight increase in the volume of the cell during stages of activity is also explained by the increased content of liquid.

According to *Weski* (1903), the nucleus is particularly rich in chromatin and is stained a darker shade when the cell has just produced its secretion.

The part played by the nucleus in the secretion has been much discussed. According to *Hertwig* (1929), it might be imagined that the nucleus either resorbs substances from the plasma in order to synthesize ferment out of these which would later on again be extruded in the plasma. Or else the raw materials would be transformed into paraplasmatic substances which would be given off to the plasma. That the nucleus would actually display an intense activity during the process of secretion was a currently adopted theory especially in earlier periods. According to *de Bonis* (1907), who examined the prostatic epithelium in dogs, the secretion emanates from the nucleus in which granules and plasmosomes are produced. Later investigators, however, have more and more begun to deny that the nucleus contributes to the formation of secretion granules (*Nassonov* 1923, 1924, *Hirsch* 1932, *Järvi* 1935, 1938 and *Ries* 1935). According to *Okkels* (1932), the nucleus is certainly involved in the process of secretion in spite of the fact that a direct substantial participation frequently does not occur.

As described above, the present investigation has shown that the nucleus is subjected to certain modifications during

secretion which obviously are closely related to the cellular secretory activity. These changes, however, are hardly of a nature to warrant us in ascribing to the nucleus a more active participation in the formation of secretion granules. On the contrary, a direct participation of the nucleus in the process of secretion was not demonstrable in these experiments.

5. *The plasma.* By plasma we here mean the ground plasma which remains if we disregard chondriome, Golgi apparatus and secretion granules. No pigment-resembling granules were observed in these prostatic epithelia.

In the cells of the prostatic epithelium in the unmated rabbit, the plasma is the least conspicuous cellular constituent. Cell-plasma is mainly seen in the most basal portions of the cell below and lateral of the nucleus. In some cases, a narrow plasma border is also observable, extending in an apical direction along the lateral walls of the cell and also a fine plasma net between secretion granules. After intense extrusion, the plasma is more distinctly observable in order to be displaced again when the cell begins to store new secretion granules.

In cells which do not produce any noticeable secretion, the plasma is fairly homogeneous and evenly eosinophile. Shortly after ejaculation when the extrusion already is considerable, the plasma may be seen to have assumed a more foamy appearance. At the same time a certain basophilic state manifests itself in the most basal parts of the cell. With increasing secretory activity, the foamy appearance of the plasma increases and the basophilia spreads over the infra- and internuclear plasma regions. 6 minutes after ejaculation the plasma is clearly foamy. The basophilia of the plasma now reaches fairly high in a supranuclear direction. With decreasing secretory intensity the plasma again assumes a more even and homogeneous structure. The basophilia also diminishes rapidly so that as soon as 9 minutes after ejaculation it is only seen as a small internuclear zone. In the following experimental stages, no basophilia was observed in the plasma.

Petersen (1909) observed that the plasma after copulation

presented a characteristic striation in the most basal portions of the cell. The foamy appearance described above revealed no signs, however, of a typically regular structure. As, moreover, it did not appear in all stages, we do not seem justified in speaking here of »Ergastoplasma« in the sense that this concept was defined by *Jacobs* (1928), that is as a purely plasmogen cellular structure composed of lamella and fibril systems, with basophile characteristics. It may be said of the plasma in the epithelial cells of the prostatic gland, as of the plasma in the epithelial cells of the seminal vesicle (*Gylling* 1938), that the basophile portions are only parts of the plasma that have changed their stainability under the influence of the increased cellular working intensity. *Macklin* and *Macklin* (1932) also thought that it depended on the functional stage of the cell whether the ground plasma was stained a lighter or darker shade. Both *Stilling* (1884) and *Walker* (1899) observed that such cells as had expelled its secretion had a smaller and lighter protoplasma. *Weski* (1903) described the large, apparently intensely secretory cylinder cell in man as having a roughly foamy protoplasma.

6. *The chondriome*. When the prostate gland has been heavily filled with secretion for a prolonged time, an opportunity arises of studying the chondriome during a stage of relative inactivity of the epithelial cells. In many cells, a small accumulation of chondriome material is then visible close to the basal wall of the cell. This conglomeration frequently reveals signs of a net-resembling structure. In some cases, however, it is not possible to observe such a basal conglomeration. The mitochondria, which depart from these conglomerations and constitute their direct continuation, in the cases where the basal conglomerations are lacking, originate directly from the immediate neighbourhood of the basal cell-wall. When the cell does not display any noticeable secretory activity, the mitochondria are as a rule very long and have a slightly sinuous course. It seems as if the mitochondria might be ramified. They develop in the longitudinal direction of the cell from the base towards the Golgi zone. Long filamentous

mitochondria, however, may also be seen in the most apical portions of the cell where they wind between the secretion granules.

In spite of the fact that no dissimilarities may be observed in the composition of the mitochondria, small drop-like swellings are visible in places. If the section has been made in a suitable place, the mitochondria may be seen to extend into the Golgi zone where they terminate in a small drop-resembling formation.

Already at the beginning of a period of intense secretory activity, the mitochondria present obvious changes. They become somewhat shorter and have numerous small drop-like swellings. The mitochondria now extend in a fairly straight line from the base of the cell towards the Golgi zone. With increasing secretory activity the mitochondria increase in number. They become still shorter and somewhat thicker at the same time as they gradually lose their uneven contour. They have a remarkably straight extension in the longitudinal direction of the cell. The chondriome now gives the impression that a strong flowing occurs from the base of the cell to the Golgi zone. 6 minutes after ejaculation the mitochondria begin again to decrease in number. They also begin to become fairly thin. Drop-like swellings are very rare except when these occur at the end parts of the mitochondria in the Golgi zone. It may be observed that the mitochondria reveal a tendency to disintegrate into short fragments arranged in a row. The basal accumulations of chondriome material are now very rare. In the most apical portions of the cell mitochondria are no longer observable. On the whole it seems as if a certain shortage of material manifests itself in the chondriome. It is now stained comparatively faintly. It seems as if the mitochondria would give off substance in the Golgi zone in the form of the small drop-like formations. In a stage of intense cellular activity this appears to occur in such quantities that the resorption of new substances in the base of the cell cannot keep pace with it.

When the secretory activity is lessened, the streaming

within the chondriome also seems to lose its earlier intensity. In the bases of the cells small accumulations of chondriome material again begin to appear. The mitochondria are fairly few in number towards the cessation of secretion, but their number increases somewhat and is soon as large as before ejaculation. Gradually, they become somewhat tortuous. The mitochondria are of a uniform width except in a few places where small drop-like swellings are again visible. The mitochondria moreover become longer. They also begin to appear apically in the cell among the secretion granules. The stainability of the chondriome gradually increases. The mitochondria appeared to be of a homogeneous consistency the whole time without different constituents being visible in them.

The occurrence of such long, filamentous mitochondria as were described above, does not agree with the observations made in the prostate gland by other writers. *De Bonis* (1907), who examined the prostatic epithelium of the dog, observed small granule-resembling »plasmosomes«. *Cowdry* (1918) also observed such granule-resembling mitochondria in the prostatic epithelium in white mice. *Moore, Price and Gallagher* (1930) examined the epithelial cells of the prostate gland in the rat and found that the mitochondria were made up of granules or small rods.

In its behaviour during the different stages of cellular activity the chondriome corresponds fairly well with the observations made on other organs by various researchers. That the number of the mitochondria increases with increasing cellular function seems to be a fairly general phenomenon (*Romeis* 1913, *Policard* 1910, *Homans* 1915, *Seecof* 1925, *Hirschlerowa* 1928, *Uotila* 1934, *Wahlberg* 1933, 1935 and *Gylling* 1938). *Dominici* (1913) also observed that the mitochondria of the prostatic epithelium vary quantitatively with the cellular activity. In active cells capable of function, the mitochondria as a rule seem to resemble long and undulatory filaments (*Regaud and Mawas* 1909, *Schultze* 1911, *Cramer* and *Ludford* 1926, *Hirschlerowa* 1928 and *Wahlberg* 1935).

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Ries (1935) and *Gylling* (1938) described how the mitochondria show a disintegration into chain- or bead-like formations in a stage of intense new-formation of secretion granules in the cell. *Busacca* (1915) and *Järvi* (1935) observed that the number of mitochondria decreased during cellular activity.

According to *de Bonis* (1907), the »plasmosomes« (by which the author evidently means the mitochondria) are formed in the nucleus in the prostatic epithelium. This view is not supported by the observations made in the present investigation. According to *Dominici* (1913), the mitochondria are not directly transformed into secretion granules. In the formation of the latter, however, the mitochondria are said to take part indirectly. The observations made in the present experiments seem rather to speak in favour of *Dominici's* theory. That the mitochondria are intimately involved in the production of secretion granules seems probable, as the mitochondria as a rule extend from the base of the cell towards the Golgi apparatus in which they may be observed to end in a small drop-like formation. Particularly at incipient intense new-formation of secretion granules, numerous such small drop-like swellings in the mitochondria are seen even in the more basal portions of the mitochondria. At the beginning of secretion the mitochondria increase in number. After the secretion has for some time been very intense, they decrease in number at the same time as they become thinner and disintegrate into fragments with fairly clear signs of shortage of material. All these facts seem to indicate rather strongly that the mitochondria in some way would be capable of delivering material to the drops of secretion. The view that the mitochondria in this manner would contribute to the production of secretion granules is in the literature supported e. g. by *Altmann* (1894), *Akutsu* (1903), *Champy* (1911), *Schultze* (1911), *Arnold* (1912), *Duesberg* (1912), *Hoven* (1912), *Maximow* (1916), *Nassonov* (1924), *Parat* (1928) and *Yamasaki* (1936). On the basis of the observations made in the present investigation it can hardly be considered as probable that the mitochondria

actually are the most important factors in the new-formation of secretion granules.

It seems extremely likely that chondriome substance is also consumed in the indirect activity of the mitochondria during secretion. Such a theory partly approaches the view that granules would in some way develop in contact with mitochondria. Suggestions in this direction have been made by *Prenant* (1910), *Laguesse* (1911), *Regaud* (1911), *Cowdry* (1918), *Emberger* (1925), *Konopacki* (1927), *Honda* (1927), *Policard* (1928), *Hirsch* (1932), *Duthie* (1934), *Järvi* (1935, 1938) and others.

Irrespective of which of these theories is the more correct, or whether both are possibly justified, facts have been adduced in the present investigation which rather clearly indicate a fairly close connection between the function of the chondriome and the new-formation of secretion granules.

7. *The Golgi apparatus.* If the intracellular granules are very numerous so that they occupy the largest portion of the cell, the Golgi apparatus is displaced towards the basal parts of the cell. If the extrusion becomes very active and the intracellular masses of granules decrease, the place of the Golgi apparatus is higher up in the cell. 6 minutes after ejaculation the Golgi apparatus reaches its most apical position and is then situated somewhat above, or in the middlemost portion of the cell. It seems as if this change in position would be fairly passive as far as the Golgi apparatus is concerned and rather be due to the general, and apparently powerful streaming within the cell from the base towards the lumen of the glandular duct. When the intensity of the extrusion again decreases and the intracellular granules become more numerous, the Golgi apparatus is again pressed down towards the base of the cell.

In these preparations, the main portion of the Golgi apparatus was always found just above the nucleus. In spite of their close topographical positions, nothing was observed that might indicate a more intimate relation between the

nucleus and the Golgi apparatus, in distinction to the observations made in thyroid cells by *Uotila* (1934).

The size of the Golgi apparatus is much dependent on the intensity of secretion. Soon after ejaculation the volume of the Golgi apparatus considerably increases. Such an increase in volume is in accordance with observations made in other organs by *Nassonov* (1924), *Kopsch* (1926), *Hirschlerowa* (1928), *Okkels* (1932), *Winters* (1933), *Uotila* (1934) and *Wahlberg* (1933, 1935). With decreasing secretion the Golgi apparatus gradually decreases so that finally it is no larger than prior to the ejaculation. *Bowen* (1926) and *Gylling* (1938) earlier described how the size and complexity of the Golgi apparatus was reduced while secretion was in progress.

In the Golgi elements of the prostatic epithelium it is also possible to observe the two substances which by *Hirschler* (1927) were termed »apparatus externum« and »apparatus internum«.

The osmiophile externum forms a network in the meshes of which the osmiophobe internum may be observed as light vacuoles. In a state of marked secretory activity, numerous small drop-like formations are visible in the apparatus externum. These may be completely surrounded by the osmium-blackened externum, but sometimes it may be observed that the black wall has thinned off and ruptured as it were so that the light drop freely communicates with the surrounding contents of the cell. In secretorily inactive stages, considerably fewer such light drops are seen in the apparatus externum.

In the light apparatus internum two constituents may be seen. The meshes in the osmiophile network are filled with grains of secretion of varying sizes from extremely small drops of the same size as the small light vesicles in the apparatus externum up to large secretion granules which greatly resemble those found exteriorly of the Golgi apparatus in the apical part of the cell. In spite of the fact that secretion granules are closely packed in the meshes of the Golgi net, they seem to be surrounded by a somewhat darker medium which might be thought to constitute the apparatus internum proper. Whether

this is of the same composition as the plasma exteriorly of the Golgi apparatus or whether it has a specific character of its own is not shown in the preparations. The secretion granules in the Golgi apparatus as a rule appear to be in the act of moving towards the apex of the cell.

In chondriome preparations, clear Golgi negatives may be observed, particularly 6 minutes after ejaculation. In synoptic preparations it is also possible to observe fairly distinct lighter areas in the Golgi zone. In inactive stages these Golgi negatives are not equally pronounced. Golgi negatives were described by *von Bergen* in the prostatic epithelium of the dog as early as 1904. *Moore, Price and Gallagher* (1930) also observed Golgi negatives in the epithelial cells of the prostate gland in the rat.

Verson (1908), *Taddei* (1910) and *Kopsch* (1926) also earlier described how the Golgi apparatus in the prostatic epithelium was composed of dark rods and loops. With regard to the functional changes of the Golgi apparatus and its significance to the secretion, the works of these authors give no information. During the study of other organs, however, many workers, as e.g. *Fuchs* (1902), *Holmgren* (1904), *Sagouchi* (1920), *Nassonov* (1923, 1924), *Bowen* (1924, 1926) and *Järvi* (1938), found signs indicating that the new-formation of secretion granules is related to the Golgi apparatus. The experiences gained in the present investigation seem to imply a very intimate interrelation between the Golgi apparatus and the production of secretion granules. It might be imagined that granules first arise in the form of small vesicles in the Golgi rods and then emerge into the meshes of the osmium-stained net and grow there. Finally, the secretion granules end up outside the Golgi apparatus in the most apical portions of the cell. It appears as if the morphology of the secretion-physiological mechanism in the prostate gland would lend support to the theory which has earlier been advanced (*Gylling* 1938) in connection with two other glands. At least the majority of the material for the secretion granules would thus be supplied by way of the chondriome to the Golgi apparatus in which

granules would then be further differentiated and developed.

According to *Nassonov* (1926, 1927), *Weiner* (1928) and *Hertwig* (1929), substances supplied from the outside are centred in the secretion granules in the Golgi apparatus.

8. *The intracellular secretion granules.* As an account has already been given of the granules situated in the Golgi apparatus, only such granules as lie freely in the cell will be dealt with in this section.

When the male has been kept in isolation for a long time and then for some time in the neighbourhood of oestral females, the cells in the prostatic epithelium are filled with closely packed secretion granules. The latter may be found even below the basally situated cell nucleus. After ejaculation the intracellular secretion granules decrease. They occur only rarely in an infranuclear position. 6 minutes after ejaculation, secretion granules are only seen, besides in the Golgi apparatus in a small zone in the most apical part of the cell. There they are no longer so closely packed. Then the number of granules increases again so that they soon occupy almost the whole of the cell. They are, however, no longer as closely packed as in the experimental stage before ejaculation. Neither do they equally frequently occur basally of the Golgi apparatus.

Petersen (1909) observed that the cells were very poor in secretion both after copulation and after injections of pilokarpin. Such basophile granules as those mentioned by *Weski* (1903) and *Petersen* were not observed in these preparations. The secretion granules always presented a clear and acidophile centre surrounded by a light, unstainable zone. This structure was most distinct in the granules situated most basally. In the granules present in the more apical portions of the cell, only the central acidophile body was mainly left. Those granules were also somewhat less distinct and very closely packed together as described above. Granules in the neighbourhood of the Golgi apparatus were stained a lighter shade by osmium than those situated in the apex of the cell. Possibly, these facts might serve as an indicator of the further development of secretion granules within the cell.

9. *The extrusion.* Before ejaculation the walls of the epithelial cells towards the lumina of the glandular ducts are very distinct and clear. If an extrusion of granules from the cell into the lumen of the glandular duct occurs at all, it is at least very difficult to observe and certainly very slow. The epithelial cells filled with granules are now in a state of relative repose. This state might be called a stage of secretion preparedness.

Soon after ejaculation, the apical cell-walls have in many places disappeared so that the contents of the cell directly communicate with the lumen of the gland. Or else it may be observed that large drops of secretion are in the process of developing at the sides of the cell-walls turned towards the lumen. 3 minutes later the extrusion is most active. Now clear apical cell-walls are only rarely observed. Then the secretion gradually decreases from stage to stage. When the present experiments were ended the extrusion was only slight.

The extrusion seems to be of purely apical nature. Its intensity appears to be almost equally great in the whole glandular epithelium. The preparations did not show how the drops of secretion pass through the wall of the cell.

Petersen (1909) found a continuous secretion in man. It seems probable that this is usually the case. Only in the special experimental stage of repletion which in the present investigation was caused in the prostate gland, the extrusion was reduced to a minimum. Eberth (1904) found that the prostate during copulation secretes the substances formed in the interval of repose. According to de Bonis (1907), Petersen (1909) and Stieve (1930), the secretory intensity of the prostatic epithelium increases during copulation, evidently even before the ejaculation. This does not seem to have been the case in the present experiments. It seems difficult to imagine that still more secretion could be contained in the prostate gland which is already filled to the full, as described above.

10. *The contents of the lumen.* Before ejaculation the lumina of the glandular ducts are heavily filled with secretion. The latter consists for the most part of a faintly eosinophile, shapeless substance. In this substance, scattered drops are

seen which reveal a perhaps somewhat more marked eosinophile state. In *Altmann-Kull* preparations, they are intensely red-coloured and finely granulated in distinction to the formless yellowish secretion. A small number of degenerated nuclei are also present.

In these rabbits, prostatic calculi did not seem to occur so frequently as described by other writers. This may possibly be explained by the comparatively young ages of these rabbits.

Shortly after ejaculation the glandular ducts contain a very small amount of secretion. They appear to be flaccid and shrunken. Then the gland is filled again fairly rapidly so that the prostate, possibly 6 minutes, but at least 9 minutes after ejaculation may be considered to hold a comparatively abundant amount of secretion.

SUMMARY.

1. In the connective tissue which supports the epithelium in the prostate gland, the erythrocytes and the content of liquid increase with the beginning of secretion and decrease in proportion as the intensity of secretion diminishes.

2. The volume of the epithelial cells increases somewhat with the beginning of secretion. Then the cells gradually decrease in size.

3. The »*Kittleisten*« are thin and distinct. Towards the end of an intense secretion, however, they become somewhat thicker.

4. In the course of secretion the nucleus is subjected to certain modifications in position, size and internal structure. These changes, however, are not of a type indicating that the nucleus is directly involved in the formation of secretion.

5. With regard to the plasma, no direct participation in the formation of secretion was noticeable. During intense secretion and extrusion, a structureless basophile area develops in the plasma. This area is formed in a zone just below the

nucleus and extends over the basal and middlemost portions of the cell. When the secretion diminishes, the basophile plasma gradually disappears.

6. The shape and mass of the chondriome is dependent on the functional stage of the cell. In normal cells, which do not exhibit a noticeable secretory activity, the mitochondria are long fragments which extend from the most basal part of the cell to the Golgi zone. At the beginning of secretion, the number and substance of the mitochondria increase. The chondriome now gives the impression that a powerful streaming occurs from the base of the cell towards the Golgi apparatus. When the secretion has been very intense for a while, signs of shortage of material may be discovered in the chondriome. The mitochondria become thinner and disintegrate into bead-like formations. When the secretion diminishes, the mitochondria resume their former appearance. When the secretion is not very active, small conglomerations of chondriome material may frequently be observed close to the basal wall of the cell. From these the mitochondria depart. When the secretion is very intense, these conglomerations disappear. They are re-formed later on when the secretion is less intense.

These observations speak in favour of an interrelation between the chondriome and the new-formation of secretion granules. Part of the function of the chondriome seems to consist in transferring material from the more basal parts of the cell to the Golgi apparatus. Regarding other characters of the chondriome, the experiences gained in the present investigation give no definite indications.

7. At the beginning of secretion, the volume of the Golgi apparatus is greatly increased. In the course of secretion, it again decreases in size until it has attained its original size. In the Golgi apparatus we may distinguish between an osmiophile apparatus externum and an osmiophobe apparatus internum. Especially when the secretion is intense, an abundance of small light drop-like formations can be seen at the externum. These may be in open communication with the in-

ternum. In the latter an abundance of granules may be observed which may vary in size from extremely small drops, resembling the small light formations in the apparatus externum, to large drops which much resemble the secretion granules outside the Golgi apparatus. Particularly in secretorily active stages, Golgi negatives may be observed in certain preparations, like a system of light discs or lamellae. In this system may be distinguished unequally large granules which were described above in connection with the Golgi apparatus.

All these facts seem to indicate fairly strongly that the secretion granules are formed in the Golgi apparatus or at least develop and grow in the latter.

8. When the extrusion is slight, the secretion granules occupy most of the cell. After very intense extrusion, granules are only present in the most apical portions of the cell. With decreasing extrusion activity, the granules again begin to fill up the largest portion of the cellular space.

9. In a state of slight extrusion the secretion drops pass through the wall of the cell in a way which was not elucidated in the present investigation. When the extrusion is very intense it seems as if the apical wall of the cell would wholly, or partly, disappear.

10. The secretion in the ducts of the prostate gland is morphologically unaltered, irrespectively of its being produced gradually or very rapidly. When the secretion is evacuated during the ejaculation, the prostate is capable of producing large amounts of new secretion within a very short time.

CONCLUSIONS.

I. When the endocrine activity of the testicles is apparently unchanged, the secretory activity in the prostatic epithelium directed towards the lumen is probably inhibited by the pressure arising between the glandular walls and the more and more increasing contents of the lumen.

II. During copulation, the ejaculation results in an intense

extrusion and production of new secretion granules in the epithelial cells.

III. The secretory activity takes place during a simple working cycle, the intensity of which is most marked at first; afterwards, it gradually diminishes.

IV. In the different experimental stages, the prostatic epithelium, and particularly the different constituents of the cell, may be observed in different secretion-physiological stages.

V. The experimental stages described above are advantageous in that they may be produced by very simple and »natural« means.

VI. The results obtained in the present investigation with reference to the prostate gland markedly agree with the experiences gained in a previous work on two other accessory sexual glands (*Gylling* 1938). In order to facilitate a comparison between these investigations, the arrangement of the present paper was made in as great accordance as possible with that of the earlier publication.

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FROM THE ANATOMICAL INSTITUTE OF THE UNIVERSITY OF
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DOCTOR OF MEDICINE AND SURGERY.

EXPERIMENTAL STUDIES ON THE NORMAL AND PATHOLOGICAL HISTOPHYSIOLOGY OF THE PROSTATE GLAND.

II. OBSERVATIONS FOLLOWING CASTRATION OF THE TEST ANIMALS.

By *P. Gylling*.

(Received for publication May 30th, 1940).

I owe a great debt of gratitude to the directors pro tem. of the Anatomical Institute, Doctors *Göran Hjelmman* and *Niilo Pesonen*, for the kind interest they have always shown in this investigation. I wish to tender my sincere thanks to Professor *Axel Wallgren* for the advise he has given me in the course of my work. I am also greatly indebted to Dr. *Martti J. Mustakallio* and Dr. *Johannes Wahlberg*. — The expenditures entailed in this work have partly been paid by a grant from *Kommerserådet Otto Malm's* donation-fund.

Numerous authors (as for instance *Rocum* 1893, *Griffiths* 1895, *Lesin* 1896, *Casper* 1897, *Athanasow* 1898, *Korenchevsky* 1930, *Leydolph* 1930, *Hansen* 1933, *Niehans* 1936) have paid attention to the changes to which the prostate gland is subjected after castration, but only *Moore*, *Price* and *Gallagher* (1930), who used rats as test animals, have taken any interest in the detailed cytological changes which occur in the prostate epithelium if the stimulating influence of the testicular hormone is eliminated.

The experiments to be described in this paper are a direct sequel to the first section of this series of investigations which is published on page 36—78 of this journal. There it was shown

that the prostate epithelium, by repletion of the lumina of the glandular ducts, could be brought into a state of comparative inactivity, or into a stage of secretion preparedness. Such a state, however, can hardly be looked upon as typical of the normal prostate epithelium in repose. An examination of the prostatic epithelium in normal animals in which the production of testicular hormone were not stimulated by external or other controllable circumstances would give an opportunity to observe the secretory activity in the prostatic epithelium under so to speak ordinary conditions. *Stieve* (1930) assumed that the prostate gland was, normally, in a state of constant, more or less intense secretion.

If the influence of the prostate-stimulating testicular hormone ceases, the prostate will atrophy according to the above-mentioned authors. In that case the secretory activity of the prostate epithelium ought also to decrease to a marked degree. If the cell portions which, in stages of secretory activity, particularly hypertrophy and show signs of activity, decrease and atrophy to an extent corresponding to the decreasing secretion, this fact would in a way constitute a proof of the close connection of these cellular constituents with the production of secretion granules.

This experimental series would thus offer an opportunity for studying a glandular epithelium in various stages of atrophy. Among the various means by which a gland can be made to atrophy, an elimination of a hormone seems to be much less harmful than if the atrophy were to be brought about by violent external interference, for instance by cutting off blood vessels or nerves, or by underfeeding the whole test animal. It therefore seems that we are justified in assuming that the atrophy of the glandular epithelium and its finest structures would here take place in as »physiological« a manner as possible.

The questions presented above may be summarized as follows:

I. What is the secretory activity of the prostate epithelium if the production of the testicular hormone in a normal test

animal is not affected by external or otherwise controllable circumstances which are calculated to stimulate this production.

II. To what change is the prostate epithelium subjected, and particularly its secretory activity, if the stimulating influence of the testicular hormone ceases.

III. Can it be established that the very cell constituents which hypertrophy during secretorily active stages, especially decrease and atrophy if the secretory activity of the prostate epithelium decreases, or ceases.

IV. Is it possible to observe in this investigation a glandular epithelium during different stages of atrophy. In that case, is the method by which the atrophy is produced especially calculated to give the latter a »physiological« character.

Material. In these experiments only rabbits were used. With regard to the standardization and characteristics of the test animals reference is made to the first publication in this experimental series (see this journal page 36). For the examination of the secretory activity in the prostate epithelium in isolated test animals, the rabbits, standardised in the manner earlier described, were kept in cages, separated from each other, in a room where no females were found. The conditions under which the animals lived were unchanged the whole time. They had to remain in these cages for a couple of months. During this time the production of testicular hormone was calculated to attain approximately the same level in all the experimental animals; this, in its turn, would result in a comparatively uniform activity of the prostate epithelium of the animals in question. Then the animals were chloroformed; the prostate portions to be examined were rapidly cut out and were immediately placed in the fixing solutions so that the sources of error which might possibly arise under narcosis or after death should be eliminated as far as possible.

4 rabbits were used in this manner. Synoptic preparations were made of one of these animals, and 3 males were used for more detailed cytological fixations.

In the experiments based on a previous castration, 33 rabbits in all were used. To render possible a comparison of the results of the investigations described in this paper and in the one previously published on the prostate gland, these rabbits, after an isolation of two weeks, were placed for two days in the neighbourhood of oestral females. Then the operation was performed in the usual manner *per scrotum*. Attention was paid to the points of view presented in an earlier discussion (Gylling 1938). After the operation the animals were kept in isolated cages until they were chloroformed. In this way preparations were taken 5 days, 10 days, 20 days, 60 days, and 6 months after castration. For the first 4 of these 5 stages, 7 rabbits were used per stage, 4 of which for cytological, and 3 for synoptic preparations. For the last stage cytological preparations were made of 2 rabbits and synoptic preparations of 3 rabbits. In this investigation 37 rabbits in all were used.

Histological technique: See this journal p. 40.

INVESTIGATIONS.

a) PREPARATIONS TAKEN AFTER THE TEST ANIMALS HAD BEEN ISOLATED AND UNMATED OVER A PROLONGED PERIOD.

These preparations resemble in many respects those which were described in a previous paper (Gylling 1940) as relating to the prostate gland after the rabbits had first been isolated for some time and then for 48 hours had been kept in the neighbourhood of females, separated from the latter only by a wide-meshed wire-netting.

The *interstitial connective tissue* which supports the epithelium of the epithelial lobes appears fairly compressed and comparatively poor in erythrocytes. The *epithelium* is single-lined, cylindric. The cells are on an average 19μ high and 5μ wide. A basal membrane may be observed in a number of preparations. Towards the lumen the cells are bounded by a mostly undulatory contour. The „*Kittleisten*“ are narrow and distinct. The *nucleus* is found in the basal portion of the cell. The nuclei are 5μ in the longitudinal direction of the cell and 5μ in the transverse direction. Their shape is usually

round. The preparations show that they are circumscribed by a distinct and unbroken nuclear membrane. 2—3 nucleoli are usually found in each nucleus. The chromatin consists of fine, well circumscribed fragments which together form a finely meshed net-work.

The *plasma* (with the exclusion of all differentiated structures like chondriome, Golgi apparatus and cell granules) seems to be rather smooth and uniform. It is very finely granulated and is stainable with eosin. The *chondriome* in some cases forms fairly small basal accumulations. The mitochondria are long with a slightly sinuous course. They extend towards the Golgi zone. The *Golgi apparatus* is found just apical of the nucleus. Its fragments, stained black with osmium, are rather thin and form together a net-work. In the individual meshes, one, or sometimes a few, secretion granules are visible. In the dark rods a number of light vesicles may also be seen.

The *intracellular secretion granules* are found from the apical part of the nucleus to the border of the cell towards the lumen of the gland. They have a darker, central grain surrounded by a light, non-stainable area. This structure of the granules is clearer the more basally they are situated in the cell. Closer to the apex of the cell only the central grains are visible. Secretion granules are seldom observed in an internuclear position and more rarely still they occur basally of the nuclei. Seemingly, the secretion granules are now no longer found in such great numbers as in the stage earlier described (Gylling 1940), when the males had been kept in the neighbourhood of oestral females.

The cells are demarcated towards the lumen by a distinct and wholly uniform cellular wall. To the side turned towards the lumen, a number of unequally large secretion drops are attached which are evidently being formed by a fusion of the intracellular secretion grains. It would therefore seem that an *extrusion* of secretion occurs from the epithelial cells in the lumen of the gland. This extrusion does not seem to be very marked, however. The *contents of the glandular lumen* consist of the usual secretion, which is mostly formed by an amorphous, faintly eosinophilic, or neutrophilic, mass, in which some drops may be discerned, being perhaps somewhat more markedly eosinophilic to judge from the preparations. A number of degenerated nuclei are also seen as well as a number of prostate calculi. The lumen of the gland is in this stage fairly well filled.

b) PREPARATIONS TAKEN 5 DAYS AFTER CASTRATION.
(See plates 1 and 2).

As short a time after the operation as this no very marked histological changes can be observed. In the epithelial cells of the gland certain cytological changes are noticeable, however. Neither in the prostate gland nor in the surrounding portions could any signs be observed, indicating an inflammation, or other ill-effects, caused by the operation.

The interstitial connective tissue. The connective tissue close to the epithelium appears to have approximately the same structure as before the operation. Erythrocytes occur very sparsely. The content of liquid seems to be comparatively low.

The epithelium. Apparently the glandular ducts are as well developed as before the operation. The epithelium, on the other hand, has become considerably lower. The cells are seemingly also somewhat more narrow than earlier. They are now on an average 17μ high and 5μ wide. The epithelium is separated from the connective tissue by an extremely thin basal membrane, which in many places is hardly noticeable. The basal cells which were earlier rather few in number have possibly increased to a certain extent.

The »Kittleisten« are as thin and clear as in the previous stage.

The nucleus in most cases occupies a basal position in the cell. Its shape is unaltered. The nuclei are on an average 5μ in the longitudinal direction of the cell and 4μ in the transverse direction. A distinct and unbroken nuclear membrane surrounds each nucleus. The number of the nucleoli has not been altered but their stainability seems to be somewhat reduced. The rods in the chromatin net hardly appear to be noticeably thicker than in earlier stages. The meshes in the net seem to be inconsiderably larger and somewhat reduced in number.

The plasma has an even and uniform character. The foamy appearance which characterized the plasma in the secretorily active stages is no longer observable. In hematoxylin-eosin preparations the plasma is rather lightly eosinophilic. In osmium-stained preparations the plasma is lighter in colour than before.

The chondriome in this stage shows a comparatively fainter stainability in most cases. Its structures have a tendency to become light and indistinct. The basal accumulations of chondriome material are either fairly small, or else they are wholly lacking. The mitochondria which depart from the basal conglomeration, or if the latter is absent, from the immediate proximity of the basal cell wall, show a comparatively tortuous course. They are fairly thin and even. The small nodes and swellings observed in normal

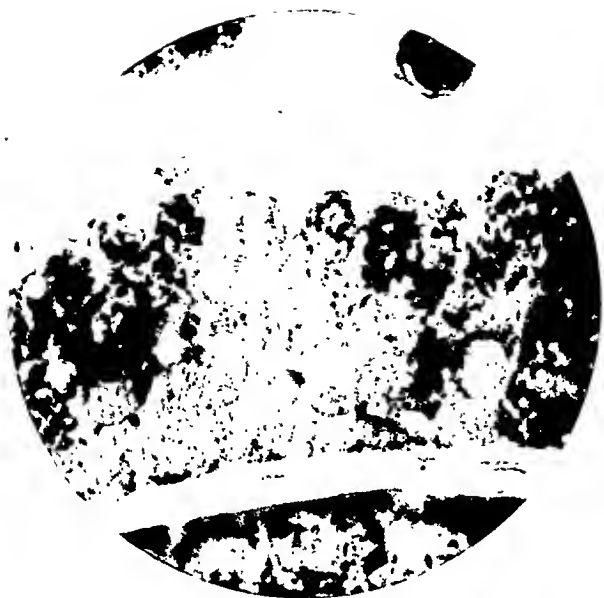


Plate 1.
Incipient epithelial atrophy. Altmann. Altmann-Kull.
Enlargement 1320.

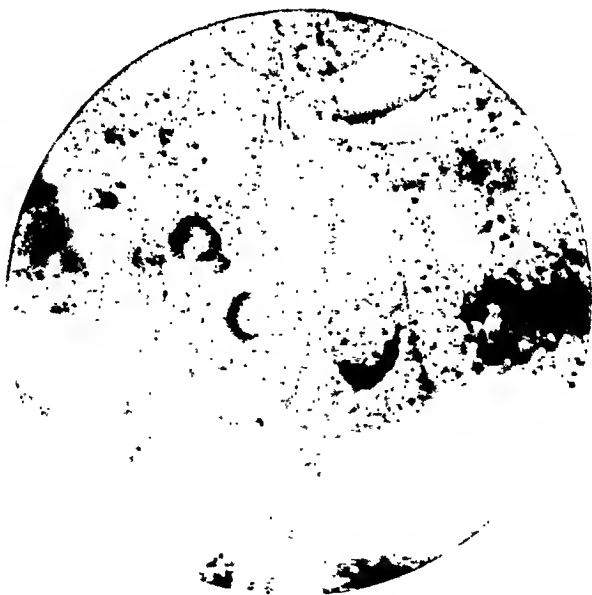


Plate 2.
Incipient epithelial atrophy. Kopsch-Kolatschew.
Enlargement 1320.

isolated and unmated rabbits are usually no longer observable. It has been described in an earlier paper how, in secretorily very active cells, the mitochondria show a tendency to disintegrate into bead-like formations. A similar tendency is noticeable here, but the fragments are now thin and of varying length. They form together undulating structures. Long and uniform, filament-resembling mitochondria also occur. The mitochondria on the whole extend in the longitudinal direction of the cell, however, from the basal part of the cell towards the Golgi zone. But it is sometimes possible to observe mitochondria which are situated in the most apical portions of the cell.

The Golgi apparatus is sometimes found immediately above the nucleus but perhaps still oftener it is situated in a more apical direction. It usually forms a more or less flattened, calotte-resembling structure, which is placed crosswise to the longitudinal direction of the cell. Other shapes may also occur. It gives the impression of being a fairly closed formation. Particularly towards the apex of the cell it is usually demarcated by a very well developed, osmium-stained rod. The net-shaped structure now appears rather incomplete in the preparations. The osmium-stained fragments are comparatively thick and lumpy. Light vesicles, of the appearance described in normal rabbits, are very rarely seen in these fragments. The line of demarcation between the osmium-stained rods and the lighter vacuoles seems to be rather diffuse. In most cases the lighter vacuoles are diffusely darker than earlier. In the vacuoles isolated secretion granules may be differentiated, however. The latter are of the same appearance as secretion granules outside the Golgi zone.

In chondriome or synoptic preparations it is hardly possible to discern Golgi negatives any more. On the whole the Golgi apparatus now gives the impression of being a fairly inactive cell constituent.

The intracellular secretion granules occupy the largest portion of the cell. It is by no means unusual to find them lateral of the nucleus and even basal of the nucleus, close to the basal wall of the cell. Their structure has been altered somewhat in that the central grains in the basal secretion granules are no longer surrounded by an equally clear and distinct, non-stainable area. The outlines of the various secretion granules also appear to be somewhat less distinct than in normal rabbits.

The extrusion is very slight. The cells are demarcated against the lumen by a distinct and uniform cell wall. As some drops of secretion can be seen to be attached to the side of the cell wall

turned towards the lumen, we must assume that a slight secretion takes place.

The contents of the lumen have the same composition as before the operation.

c) PREPARATIONS TAKEN 10 DAYS AFTER CASTRATION.

(See plates 3 and 4).

The interstitial connective tissue. The connective tissue which separates the different prostatic ducts has become thicker. The connective tissue supporting the epithelial lobes projecting into the lumen have also become thicker. The structure of the connective tissue appears to a certain extent to be more compact. Erythrocytes are comparatively rare close to the epithelium. In the small blood vessels which are found at a certain distance from the epithelium, numerous red blood corpuscles are visible, however. The content of liquid in the connective tissue appears to be still more reduced than in the preceding stage.

The epithelium. The lumina of the prostatic ducts have become somewhat smaller. The epithelial lobes have become a little shorter and wider than before. It becomes increasingly rare to find thin epithelial lamellae extending across the lumen of the glandular duct. The epithelium has become longer and narrower. The cells are now 14μ high and 4μ wide on an average. Basal cells occur somewhat more densely. It is now difficult to discern the basal membrane which earlier separated the epithelium from the connective tissue.

The »Kittlcisten« are possibly somewhat thinner but very distinct.

The nucleus. The nuclei lie close to the basal walls of the cells. They are usually round but may adapt themselves to the shape of the cell. For instance, high, narrow cells may present oval nuclei placed in the longitudinal direction of the cell, and flat cells may present nuclei somewhat flattened towards the base of the cell. It is rather difficult now to give measurements of the average dimensions of the nuclei. As some sort of mean value we may give 4μ for the height of the nuclei in the longitudinal direction of the cell and 3μ for the width. The nuclei are surrounded by a distinct and uniform nuclear membrane. The nucleoli stain rather badly. The fragments of the chromatin net are, relatively speaking, slightly thicker and more lumpy. The meshes in the chromatin net are similarly perhaps somewhat larger.

The uniform character of the *plasma* here appears still more



Plate 3.

Fairly clear epithelial atrophy. Altmann. Altmann-Kull. Enlargement 1320.

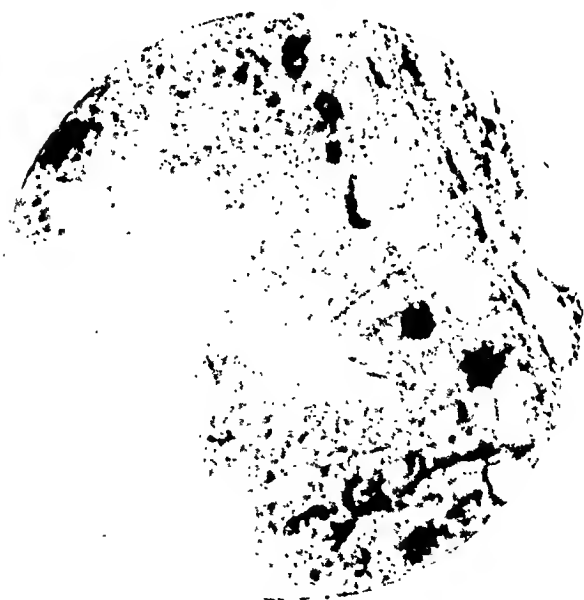


Plate 4.

Fairly clear epithelial atrophy. Kopsch-Kolatschew. Enlargement 1320.

distinctly. The plasma stains more lightly eosinophilic than in the preceding stage. In osmium-stained preparations the plasma is also very light.

The *chondriome* is greatly reduced. In most cells the basal accumulations of chondriome material are wholly lacking. In other cells it is possible to discern small rudiments of the basal conglomerations. The mitochondria depart from the base of the cell and still extend in its longitudinal direction. They are somewhat less numerous than earlier. Long filamentous forms are not so frequent. As a rule the mitochondria are made up of short thin rods of unequal length. Granule-resembling mitochondria, placed in a row, may also occur. The mitochondria have in most cases a straight, non-tortuous course. Above the Golgi zone they are not discernible with certainty.

The chondriome stains more faintly than in the preceding stage, its outlines are comparatively indistinct.

The *Golgi apparatus*, as in the earlier stage, lies either closely apical of the nucleus but more frequently somewhat closer to the apex of the cell. Its volume has been considerably reduced. It has a rather compact appearance and seems to be a fairly closed formation in all directions. In the massive and lumpy, osmium-blackened rods, small light vesicles are very rarely seen. The lighter vacuoles between the rods are also small and few in number. They seem to be darker than the medium surrounding the Golgi apparatus. Whether secretion granules are present in these lighter vacuoles or not is difficult to observe in the preparations. Golgi negatives are hardly visible in Altmann-Kull or other preparations.

The *intracellular secretion granules* still occupy a large portion of the cell. They are mostly present in the more apical portions of the cell, but it is by no means unusual to observe secretion granules also close to the basal wall of the cell. The light area which surrounds the more basal secretion granules does not appear quite as clearly as before. The secretion granules have become smaller and stain somewhat less intensely than earlier. They also seem to occur fairly sparsely in the cell as compared to previous stages.

The *extrusion* seems to be very slight to judge from the few and inconsiderable secretion drops which are attached to the side of the apical cell wall which is turned towards the lumen. Nevertheless, a certain secretory activity seems to occur.

The *contents of the lumen* seem to have become somewhat reduced. Their composition, however, appears to be about the same as earlier.

d) PREPARATIONS TAKEN 20 DAYS AFTER CASTRATION.
(See plates 5 and 6).

The interstitial connective tissue. The connective tissue between the different glandular ducts is now considerably thicker as compared to the rest of the gland. It consists of compact masses of fibrils of connective tissue. The nuclei have increased in the layer of connective tissue bordering on the epithelium. Fairly numerous small blood vessels, filled with erythrocytes, are also seen here. In spite of this, the content of liquid in the connective tissue appears to be rather low. The connective tissue which supports the lists of epithelium projecting into the lumen has also become broader and more compact.

The epithelium. The lumina of the glandular ducts have become still more reduced. Ducts are also visible in which the lumen has become filled up altogether. The epithelium has now almost wholly lost its earlier character. There are frequently two or more layer. The cells are small and may assume every shape from low-cylindric and cubic to flattened. As an approximate mean for the cells bordering on the lumen may be mentioned 6μ for the height and 4μ for the width. No basal membrane separating the epithelium from the connective tissue can be discerned. In many places it rather looks as if the epithelium passes into the connective tissue gradually and imperceptibly.

The »Kittelleisten« are still very thin and distinct. They form together a net which is somewhat thicker at the corners.

The nucleus occupies the largest portion of the cell. On account of the small size of the cells, the nuclei usually lie in about the middle of the cell. If a cell has kept its cylindric shape, the nucleus is found closer to the base of the cell. The shape of the nuclei is the same as in the preceding stage. An approximate mean for the height of the nuclei is 4μ and for their width, 3μ . They are surrounded by a distinct nuclear membrane. The nucleoli hardly stain differently from the chromatin. The chromatin fragments are comparatively thicker and lumpier than before. The light vacuoles in the chromatin net are also, relatively speaking, rather large and few in number.

The plasma which surrounds the nucleus in a thin layer, of fairly uniform thickness, resembles the one seen in the previous stage, being lightly eosinophilic. In osmium-stained preparations the plasma is very light.

The chondriome is now very strongly reduced. It stains very faintly and indistinctly with oxygen-fuchsin. In the plates it is difficult to discern even parts of the chondriome. At a close study of the preparations it is possible, especially in cells which have

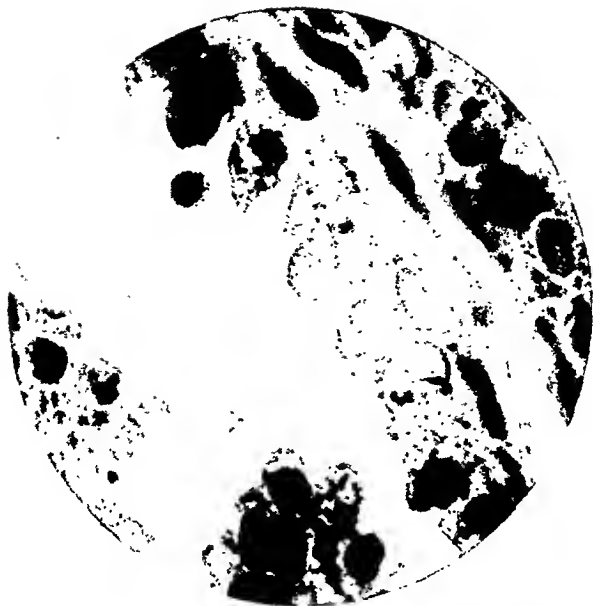


Plate 5.
Clear epithelial atrophy. Altmann. Altmann-Kull.
Enlargement 1320.



Plate 6.
Clear epithelial atrophy. Kopsch-Kolatschew.
Enlargement 1320.



Plate 7.

Marked epithelial atrophy. Altmann. Altmann-Kull.
Enlargement 1320.



Plate 8.

Marked epithelial atrophy. Kopsch-Kolatschew.
Enlargement 1320.

better preserved their cylindric shape, to observe scattered granules in the vicinity of the basal wall of the cell. Sometimes it is also possible to discern thin and faintly stained filaments which usually extend in the longitudinal direction of the cell. They may, however, extend in any direction within the cell.

The volume of the *Golgi apparatus* is still more reduced. It is frequently found closer to the nucleus than earlier. It is, however, possible, in cells which have preserved their cylindric shape, to observe Golgi apparatuses the position of which is considerably more apical. In the latter position the Golgi apparatus often assumes an arch-resembling shape, with the convexity towards the lumen of the prostatic duct. This shape also occurred in the previous stage. If the Golgi apparatus lies closer to the apical pole of the nucleus, the osmium-blackened fragments compose a more irregular formation. The fragments are thick and lumpy. No vesicle-shaped structures are visible in them. It is rare to find that the rods form lighter vacuoles between them. If such vacuoles occur, they are small and fairly dark. No observation can be made showing that they contain secretion granules. No Golgi negatives are visible in the preparations.

The intracellular secretion granules are mostly found in the most apical portions of the cell. Scattered granules are visible, however, in any other portions of the cell. They are considerably smaller in volume than formerly. The lighter area round the central grains of the secretion granules is very indistinct. Granules occur relatively sparsely as compared to the earlier stages.

The extrusion is hardly noticeable. It is possible, however, to discern in some places small drops, or rather secretion fragments, which are attached to the outer side of the apical cell wall. It is most reasonable to assume that this substance has been discharged from the epithelial cells fairly recently.

The contents of the lumen have become further reduced. It is difficult to decide to what extent their composition has been altered. It seems as if the secretion in the lumen were almost wholly composed of an amorphous substance of the same stainability as before.

c) PREPARATIONS TAKEN 60 DAYS AFTER CASTRATION.

(See plates 7 and 8).

The interstitial connective tissue. The different glandular ducts are separated by a comparatively thicker layer of connective tissue than before. The connective tissue of the epithelial lists projecting into the lumen has also become thicker and the epithelial lists are

shorter. Erythrocytes are very rarely observed. The connective tissue gives everywhere a very massive impression.

The epithelium. Only part of the glandular lumina are preserved. These are narrow and greatly reduced also in length. Other glandular ducts have been wholly filled up so that they look like compact strings made up of epithelial cells clustered together. The epithelium as a rule has several layers and frequently lacks a sharp line of demarcation towards the connective tissue. The shape of the cells may vary within rather wide limits. Usually the cells are small and low. As an approximate mean for the cells close to the lumen may be mentioned 4μ for the height and 5μ for the width.

The »Kittleisten« are very thin but nevertheless distinct.

The nucleus. The nuclei lie in the middle of the cell surrounded by a very thin layer of plasma. Their shape as a rule adheres to the shape of the cell. On an average they are 3μ high and 4μ wide. In the preparations they are demarcated by a clear and unbroken outline. The number of nucleoli is still the same as formerly. Their stainability is poor. The chromatin consists of fairly thick, lumpy fragments surrounding comparatively large, light vacuoles.

The plasma is uniform and stains faintly eosinophilic. In osmium-stained preparations the plasma is very light.

The chondriome is now extremely reduced. It stains faintly with oxygen-fuchsin. The only visible parts of the chondriome are a few scattered granule-resembling mitochondria in the narrow plasma belt which surrounds the nucleus. The majority of such mitochondria are found in the neighbourhood of the basal wall of the cell. In exceptional cases it is possible to observe isolated mitochondria in the shape of short thin rods. These do not extend in any particular direction in the cell to judge from the preparations.

The Golgi apparatus is completely rudimentary. It consists of a small black lump which mostly lies just apical of the nucleus. Often it lies lateral of the nucleus, around which it forms an incomplete girdle. Neither in the black lumps nor between them is it possible to discern any lighter vacuoles. No Golgi negatives are visible.

The intracellular secretion granules are very few in number. Most of them are found close to the border of the cell towards the lumen but in other parts of the cell isolated secretion granules can also be observed in some cases. They are very small and inconsiderable. Sometimes it is possible to observe that they are indistinctly composed of a darker centre surrounded by a thin, somewhat lighter area.

The extrusion. In the preparations an extrusion can hardly be

observed any longer. If the small quantities of substance which may sometimes be observed to adhere to the side of the apical cell walls turned towards the lumen, are to be looked upon as recently secreted from the cells, a very slight extrusion is present in this stage, too.

The contents of the lumen seem to be very scanty. They consist of an amorphous substance which is perhaps somewhat thinner and more eosinophilic than before. Prostate calculi may still be observed. A comparison between the calculi in this stage and in normal rabbits shows that the calculi now seem to be as a rule smaller in volume. Can this circumstance be a sign that the calculi are actually subjected to a process of disintegration?

With regard to the material used in these and previous histophysiological studies of the prostate gland it should be pointed out that the animals were evidently so young that large prostate calculi have not yet had time to develop. The number of stones was fairly small. It is therefore very probable that the observation to the effect that the prostate calculi are reduced in volume in animals which have been castrated over a prolonged period, should rather be looked upon as hypothetical.

f) PREPARATIONS TAKEN 6 MONTHS AFTER CASTRATION.

These preparations greatly resemble those taken in the preceding stage. The atrophy of the glandular ducts of the prostate has further advanced somewhat. The different glandular ducts are separated by a thick and compact *connective tissue*. Close to the epithelium, isolated small blood vessels may be observed which contain a fair abundance of erythrocytes. The *epithelium* has many layers and consists of a muddle of small cubic or flat cells. In many glandular ducts the lumen is wholly filled up. Between the cells portions of a fairly clear net of »Kittleisten« may be seen. The *nuclei* occupy the largest portion of the cell. Their shape is adapted to that of the cell. They behave as in the previous stage. The same applies to the *plasma*. The *chondriome* is extremely reduced. It consists of a few isolated, scattered grains in the more basal portions of the cell. The *Golgi apparatus* consists of a wholly osmium-blackened small lump which is usually situated just apical of the nucleus or sometimes has a somewhat more basal position close to the nucleus. The *intracellular secretion granules* are very few in number and small. They are situated in the most apical portions of the cell. Judging from the thin, faintly eosinophilic secretion which makes up the *contents of the lumen*, a very slight *extrusion* seems to take place in this stage as well.

DISCUSSION.

1. *The interstitial connective tissue* which supports the prostatic epithelium and surrounds the separate glandular ducts appears to be rather compressed when the test animals have long been isolated and unmated. Shortly after the castration, conditions are the same, but as soon as 10 days afterwards the connective tissue may everywhere be seen to have become thicker as compared to the rest of the organ. The connective tissue seems to be more compact, with a lower content of liquid. Close to the epithelium, capillaries containing erythrocytes are few in number, but in the small blood vessels, at a certain distance from the epithelium, numerous red blood cells are found. These changes become still more distinct when a longer period has elapsed after the castration. The nuclei in the connective tissue close to the epithelium become more numerous. If a long time has elapsed after the castration, the connective tissue in the atrophied organ is comparatively predominant. A similar observation was also made by *Moore, Price and Gallagher* (1930) in rats. According to *Leydolph* (1930), the connective tissue was also increased in the neighbourhood of the glandular ducts. The structure of the connective tissue is compact and its content of liquid is low. Close to the epithelium isolated small blood vessels may be seen, containing erythrocytes.

2. *The epithelium.* After the castration the glandular ducts become shorter and smaller. Epithelial lamellae extending across the lumina of the ducts no longer occur. Often the glandular ducts are wholly filled up if a long time has elapsed after the castration. The epithelium, which under ordinary circumstances is single-lined and high-cylindric, is subject to a marked change after the castration. Shortly after castration, the size of the epithelial cells is greatly reduced. The cellular shape becomes increasingly lower so that the cubic or flat epithelial cells finally predominate. The basal cells increase to a certain extent. In opposition to *Schaap* (1899), *Leydolph* (1930) refuses to admit the presence of basal cells after

castration. At the same time as the single-lined epithelium gradually changes into an epithelium of several layers, the basement membrane becomes more and more indistinct and finally disappears altogether. The borderline between the epithelium and the subjacent connective tissue becomes increasingly difficult to observe. According to *Griffiths* (1895) and *Macklin* and *Macklin* (1932), the epithelium is subjected to a fatty degeneration, the cells are liable to lose contact with the basement membrane and land up in the secretion in the lumen of the glandular duct. I have made no observation showing that the epithelium disappears altogether (*Casper* 1897, *Athanasow* 1898, *Niehans* 1936). In these investigations, ejected cells or remnants of cells were only in exceptional cases visible in the lumina of the glandular ducts. The atrophy of the cell takes place at a comparatively quick rate up to 20 days after castration; then the cells continue to atrophy at a considerably slower rate. 6 months after castration the epithelium of the glandular ducts is composed of a muddle of small flat or cubic cells in many layers. Wholly filled up glandular ducts are not infrequent. No reduction in the number of glandular ducts has been observed.

3. *The »Kittleisten«* in the prostatic epithelium of the isolated and unmated rabbit are small and distinct. They form together a net which is somewhat thicker at the corners. After castration the »Kittleisten« gradually become narrower but are the whole time very distinct. *Athanasow* (1897) and *Leydolph* (1930) described how the borderlines between the epithelial cells become diffuse after castration.

In these experimental stages no observation was made showing a division of the »Kittleisten« similar to the one described for secretorily more active stages (*Gylling* 1938, 1940).

4. *The nucleus.* In the normal prostatic epithelium the nuclei lie basal of the epithelial cells. According to *Petersen* (1909), the nuclei had also a wholly basal position when the test animals had long been kept in isolation. If the supply of testicular hormone ceases, the cells decrease to a higher

degree than the nuclei, which gradually occupy a more central position. Finally the nucleus occupies the largest portion of the cell.

The shape of the nuclei usually adapts itself to the shape of the cell. In the normal, high-cylindric prostatic epithelium, the nuclei are usually rather spheric, but in the flat cells occurring after castration the nuclei may be drawn out in a transversal direction. The volume of the nuclei is only reduced to a comparatively small degree in relation to that of the cells.

The nuclei are always surrounded by a distinct and unbroken nuclear membrane. A discharge of visible nuclear constituents into the medium surrounding the nucleus could in no case be observed. In all the experimental stages nucleoli were visible in the nuclei. They were usually 2—3 in each nucleus. After a long time of castration they were sometimes fairly difficult to observe. *Leydolph* (1930), as long time after the castration as 17 months, noticed nucleoli in the nuclei of the prostatic epithelium of the rabbit. *Moore, Price and Gallagher* (1930), on the other hand, were unable to find any nucleoli in rats after a time of castration of 20 days. These workers also noticed distinct degenerative changes in the chromatin net of the nuclei, especially after prolonged castration. The fine, distinctly circumscribed chromatin fragments, which together formed a finely meshed network before the castration, were quite considerably changed after the supply of testicular hormone had ceased. They assumed an increasingly lumpy and thick appearance, while the meshes in the net became comparatively large and light. Finally the chromatin was composed of lumpy fragments which were surrounded by comparatively large, light vacuoles.

No observation could be made indicating that the nucleus visibly participated in the cell work.

5. *The plasma.* By plasma we here mean the plasma which remains if we disregard chondriome, Golgi apparatus and secretion granules. Pigment-resembling granules were not observed in these prostatic epithelia.

The plasma which in a normal prostatic epithelium fills up the scanty room between the intracellular secretion granules, is eosinophilic and has an even, finely granulated appearance. After the castration, the cell plasma is strongly reduced and finally surrounds the nucleus in a very thin layer. The longer the time that has elapsed after the castration, the lighter and more uniform is the character of the plasma. *Leydolph* (1930) observed that the plasma in the prostatic epithelium of the rabbit was homogeneous and stained fairly strongly 17 months after castration. The foamy appearance or granulation which characterizes the plasma of the prostatic epithelium in the secretorily active stages (*Gylling* 1940) can no longer be observed. *Moore, Price and Gallagher* (1930), in the prostatic epithelium of the rat, noticed that the plasma, 10 days after castration, had become homogeneous and more lightly staining than earlier.

6. *The chondriome.* During the course of the present investigation an excellent opportunity presented itself for studying the chondriome during different stages of atrophy. In the prostatic epithelium of normal test animals which had long been isolated and unmated, it was possible, in some cells, to observe small basal accumulations or conglomerations of chondriome material of the same appearance as has earlier been described (*Gylling* 1940). This basal accumulation becomes smaller the longer you leave the animals after castration before taking the preparations.

The mitochondria depart from this accumulation of chondriome, or, if the latter is lacking, from the immediate neighbourhood of the basal cell wall. The long, slightly tortuous mitochondria soon after the castration assume a tendency to disintegrate into longer or shorter fragments. Gradually, the mitochondria, which become more and more reduced in number, are composed of increasingly shorter, thin rods. Above the Golgi apparatus no mitochondria were observed in these experiments. As soon as 20 days after castration, the chondriome is very markedly reduced. The mitochondria are composed of some isolated granules, which lie scattered

in the plasma around the nucleus, particularly in the region of the basal wall of the cell. When a more prolonged time has elapsed after castration, this place is the only spot in which the more and more infrequent mitochondria can be observed.

The chondriome which in normal cases clearly tends to extend in the direction from the basal wall of the cell towards the Golgi zone shortly after castration shows signs of a certain disorientation in that the fragmentary mitochondria mutually form undulating formations. After a prolonged interval following the castration an extension of the chondriome in a definite direction within the cell can no longer be observed. *Weatherford* (1933) undoubtedly is right in stating that »the chondriosomes are early and sensitive indicators of cellular change and injury.«

The flowing which has been observed in the chondriome in secretorily active stages (*Gylling* 1938, 1940) could not be observed in the present experiments. The mitochondria seem to be of homogeneous consistency. The stainability of the chondriome decreases the longer time that has elapsed after castration. This fact might possibly indicate changes in the composition of the chondriome.

Moore, Price and Gallagher (1930) were unable to observe distinct changes in the mitochondria in the prostatic epithelium of the rat after castration. The changes described above, however, correspond well with *Wahlberg's* (1935) statement to the effect that the chondriome is subject to degenerative changes before nucleus and plasma in the cell. *Okkels* (1932) also states that the chondriome is very susceptible to cell injuries.

The fact that the mitochondria simply disintegrate into smaller and smaller portions is evidently due to the origin and nature of this glandular atrophy, in the process of which the stainability of the chondriome becomes reduced and partly ceases. The more usual form of atrophy within the chondriome seems to consist in the mitochondria at an early stage disintegrating into lumps of various shapes and melting together

into ring-shaped formations (Castrén 1923, 1926, Chydenius 1926, 1929, v. Numers 1932).

7. *The Golgi apparatus.* After the influence of the testicular hormones has been reduced, or altogether ceased, the Golgi apparatus passes through a series of typical changes. In prostatic epithelium from rabbits which have long been isolated and unmated, the Golgi apparatus lies just above the nucleus as a net-resembling formation. Shortly after the castration the position of the Golgi apparatus is somewhat more apical in the cell. Its volume is noticeably reduced during this time. With increasing atrophy of the cell, the position of the Golgi apparatus becomes again closely apical as related to the nucleus. A very long time after castration the Golgi apparatus may be found lateral of the nucleus around which it forms an incomplete girdle. The displacements of the Golgi apparatus seem to be wholly passive, due to the advancing atrophy of the cell. Moore, Price and Gallagher (1930) also described how the volume of the Golgi apparatus became reduced and how, finally, it only consisted of some fragments situated to one side of the nucleus.

According to Hirschler (1927), two constituents may be distinguished between in the Golgi apparatus: an osmiophilic apparatus externum and an osmiophobic apparatus internum. Before the castration it is possible to observe in the Golgi apparatuses of the prostatic epithelium how the osmiophilic part, in the form of fine loops, composes a typically net-shaped formation. In these osmium-blackened rods, isolated light areas are visible. As has earlier been pointed out (Gylling 1938, 1940) it is probable that these light drop-like formations are intimately connected with the formation of new secretion drops in the cell. After the castration these light formations become more and more infrequent and can no longer be observed at all, if the preparations are taken after the influence of the testicular hormone has been absent over a prolonged period. The light meshes in the net formed by the apparatus externum compose the osmiophobic apparatus internum. Before castration these meshes are wide; and they may be seen

to contain isolated, or several, secretion drops of various sizes, having the same appearance as the intracellular secretion drops situated more apical in the cell. After castration the light meshes become increasingly smaller and it is particularly difficult to discern any drops of secretion in them. Finally, the apparatus internum wholly disappears and only compact osmiophilic fragments may be observed. In this way the Golgi apparatus assumes a more and more closed appearance.

Before the castration it is possible, especially in Altman-Kull preparations, to observe typical clear, colourless areas in the Golgi zone. These Golgi negatives may sometimes be indistinctly discerned shortly after castration, but afterwards disappear altogether. This was considered by *Moore, Price and Gallagher* (1930) and by *Hansen* (1933) to be a very typical degenerative change.

The above shows that the Golgi apparatus becomes simpler and more and more reduced in proportion with the reduction in the number of secretion granules that surround it.

8. *The intracellular secretion granules.* In this section shall only be dealt with the granules which lie freely in the cell, as an account has been given above of the secretion granules in the Golgi apparatus.

If the test animals have been isolated and unmated over a prolonged period we may observe that the apical portions of the cells of the prostatic epithelium become for the most part filled with grains of secretion. The latter may partly surround the sides of the nucleus but they seldom appear between the nucleus and the basal cell wall. *Petersen* (1909), with reference to a similar stage in the rabbit, described how the plasma was everywhere crammed with large, acidophilic grains. After the castration the largest portion of the cell is occupied by secretion granules but these cannot be observed to extend in a definite direction. Gradually the secretion granules begin to disappear from all the other parts of the cell except close to its apical wall. Finally, only isolated secretion granules remain close to the apical wall of the cell.

Especially in the secretion granules which are situated

more basally it is possible, before castration, to observe a strongly stained centre, surrounded by a light non-stainable area. After castration, the light area gradually becomes more and more indistinct at the same time as the secretion drops become smaller and their centre more faintly staining. A long time after the castration the secretion grains only consist of a small, faintly stainable drop.

9. *The extrusion.* The cells of the prostatic epithelium in the unmated normal test animal show a uniform, undulatory outline towards the lumina of the glandular ducts. To the surface of these apical cell walls which is turned towards the lumen, a number of unequally large secretion drops are attached which are evidently secreted from the epithelial cells. This observations agrees with the assumption made by *Stieve* (1930) to the effect that the epithelial cells in the normal prostate are in a constant secretory activity. It seems as if this secretion would take place at a fairly slow rate, however. *Röhlich* (1938) could state the same in man.

After castration the extrusion decreases gradually and is, finally, hardly noticeable. The apical wall of the cell is never broken by escaping secretion, as was the case in the secretorily very active stages which have earlier been described (*Gylling* 1940).

10. *The contents of the lumen.* Before the castration, the lumina of the glandular ducts are fairly well filled with the typical prostate secretion. After castration the quantity of this secretion becomes gradually reduced. The secretion gradually assumes a thinner consistency, as it were. It has a faintly eosinophilic character. It seems probable that the prostate calculi become somewhat smaller after prolonged glandular atrophy.

SUMMARY.

1. After castration the connective tissue becomes relatively thicker and more compact. Its content of nuclei is increased.
2. After castration the epithelium is rapidly subjected to

great changes. The volume of the high-cylindric cells is strongly reduced and their shape approaches more and more the cubic or flat type. The originally single-lined epithelium in many places changes into one of several layers.

3. The »Kittleisten« are always distinct. After castration they become somewhat thinner.

4. After castration the volume of the nucleus is slightly reduced in relation to the reduction in the volume of the cells. The stainability of the nucleus decreases and some degenerative changes may be observed in its inner structure.

5. The structure of the plasma becomes increasingly even and uniform after castration. It stains lightly eosinophilic.

6. When the secretory activity of the cell decreases after castration, the chondriome is also reduced. Shortly after the castration the long undulatory mitochondria disintegrate into unequally long, consecutively lined fragments. The mitochondria thus become increasingly shorter and at the same time their stainability decreases and their number becomes reduced. Finally the chondriome is only composed of a few isolated, faintly stained granules. After castration, the mitochondria show signs of disorientation in the cell.

7. The Golgi apparatus is subjected to changes corresponding to those occurring in the chondriome. The volume of the Golgi apparatus is considerably reduced. Its position, which shortly after castration is somewhat more apical, gradually approaches the nucleus, or even the side of the nucleus. The osmiophilic apparatus externum strongly decreases. The osmium-stained fragments become thicker and more compact. The osmiophobic apparatus internum finally disappears altogether. The secretion drops which, especially before castration, frequently occurred both in the apparatus externum and in the apparatus internum, after castration are extremely difficult to discover, particularly when a prolonged period has elapsed after the operation.

8. The intracellular secretion granules strongly decrease in number. Their volume becomes reduced and so does their stainability. The light, non-stainable area which in normal

cases surrounds the central, strongly stained centre in the secretion granules gradually disappears.

9. In the prostatic epithelium of the unmated normal rabbit a slow, even extrusion apparently takes place. A certain extrusion evidently also occurs after the castration. The present experiments did not throw any light on the mechanism involved in the passage of the secretion through the apical wall of the cell.

10. After castration the lumina of the glandular ducts are filled with a scanty, amorphous secretion which is faintly eosinophilic and which has a distinctly thinner consistency than in normal cases.

CONCLUSIONS.

I. If no special external circumstances stimulate the production of testicular hormone in the normal test animal, the prostatic epithelium is in a state of slow, proceeding secretion.

II. After the stimulating influence of the testicular hormone on the prostate gland has ceased, there sets in a comparatively rapid atrophy of this gland, particularly of its epithelium. In this process the secretory activity of the prostatic epithelium is also reduced to a very high degree.

III. The experiments show that the very cell constituents which were particularly active in stages of secretion, atrophy most rapidly when the secretory activity of the prostatic epithelium decreases in intensity.

IV. These experiments have offered an excellent opportunity for studying different stages of atrophy in a glandular epithelium. This atrophy was brought about by cessation of a hormonal influence. The method may be looked upon as sparing the organ quite considerably; seemingly the atrophy takes place in as »physiological« manner as possible.

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PHAGOCYTOSIS EMPLOYED AS A SEROLOGICAL TEST IN BRUCELLOSIS.

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In Brucellosis an active infection now and then has been determined in individuals, giving a negative agglutination test with bac. Ab. Bang. On this account it has been the constant endeavour to find a sensitive reaction, which could be used in connection with the agglutination- and complementfixation-test.

Huddleson³⁾ published a cytophagic reaction which is now in wide use in America. This reaction requires the patient's blood to which is added a small quantity of sodium citrate. The test must be made within six hours after drawing the blood, and during this time the sample must be kept in a chilled state in order to preserve the cytophagic property of the leucocytes. Equal parts of the blood and of a bacterial suspension are mixed together and placed in a waterbath at 37° C. for half an hour, whereafter spreads are prepared on glass slides. After staining it is possible to see to what degree the phagocytosis has proceeded. (For technical details, see Huddleson²⁾).

From this reaction Huddleson draws the conclusion that the phagocytic power of the leucocytes in patients with an active, fresh infection (and positive agglutination test) is only small, but that it increases as soon as the patient begins to recover. He assumes that the immune-opsonins do not appear till a late phase of the disease, and makes use of this »absence of phagocytic power«, viewed in conjunction with

the positive agglutination test and positive skin test, to diagnose an active infection. At a later phase of the disease, when the phagocytic reaction has become positive, Huddleson considers the patient to be immune.

It is a recognized fact, that during the course of an infectious disease, different antibodies do not all develop at the same time. For instance, the complement-fixing antibodies in *Brucella* infection sometimes make their appearance at a later phase of the disease than the agglutinins, and they persist longer (M. Kristensen and P. Holm).⁴⁾

Having regard to what is generally known concerning the behaviour of the opsonins, it would seem peculiar that in Brucellosis the opsonins should occur at a much later point of time than both agglutinins and complement-fixing antibodies.

In my investigations into the content of immune-opsonin in the blood in Brucellosis I have arrived at the conclusion that these antibodies occur at a very early phase of the disease, most frequently before the agglutinins. That the same conclusion cannot be drawn when employing Huddleson's method, however, is solely on account of the technique employed; in the following I shall therefore describe the method I have used in the cythophagic reaction, whereby it is seen that the opsonin content of the blood is high at a very early phase of the disease — at any rate always when the agglutination test is positive.

Technique.

Instead of using the patient's citrated blood and observing the phagocytosis in the leucocytes, the author's method is to use the patient's serum. Phagocytosis proceeds on the addition of citrated blood not containing *Brucella* opsonin, taken from a donor previously tested for this and stationed at the Institute, so that the blood can be used immediately after being collected. 5 c. c. blood is taken from an arm vein by means of a syringe and mixed in a tube with 0,2 c. c. 20 per cent sodium citrate ($C_6H_5O_7Na_3 + 5\frac{1}{2}H_2O$).

The patient serum heated at 56° C. for half an hour is transferred with a capillary pipette to the bottom of a small testtube.

The quantity used is 0.02 c. c. To this is added 0.1 c. c. bacterial suspension of the same density as that used by Huddleson (1 cm. by the Gates apparatus). The tube is shaken and placed for half an hour in a water-bath at 37° C., whereafter 0.1 c. c. of the aforesaid non-phagocytic citrated blood is added. The tube is shaken again and replaced in the water-bath at 37° C. In the next half hour, when phagocytosis is proceeding, the tube is shaken at least four times. With a pasteur pipette a small drop is then placed on one end of a clean (non-greasy) slide, whereafter the spread is made by means of a plane slide without corners. As the viscosity of this mixture is less than that of ordinary ear blood, it is important that the glass used spreading is held at right angles to the slide, otherwise all the leucocytes collect at one end.

The slide is air-dried, hardened for two minutes in a concentrated sublimate solution, thoroughly rinsed and then stained with aqueous methylen-blue solution, 0.2 %, for ten minutes. It is then rinsed again, but not too vigorously in order not to make the stain too weak.

Estimating the cytophagic activity of the leucocytes proceeds exactly as described by Huddleson (25 leucocytes are counted, and a distinction is made between maximum phagocytosis: the cell contains over 40 bacteria, moderate phagocytosis: the cell contains from 21 to 40 bacteria, slight: 1 to 20 bacteria, negative: 0 bacteria. Each reaction is given four numbers; that to the left means the number of leucocytes with maximum phagocytosis, the number to the right the total of non-phagocytic leucocytes, and those in between represent moderate and slight phagocytosis respectively).

When testing serum that gives a positive agglutination or complement-fixation reaction, the aforesaid serum dosis should be diminished so that a tube containing 0.02 c. c. of a 1:10 serum dilution should be used in addition. This should also be the case when there is a positive phagocytic reaction in the highest serum concentration.

This method presents the following advantages:

1. The blood specimens can be taken in the usual manner without any addition; they can be forwarded, and they need not be tested within six hours.

2. Serum titration provides an inkling of the quantity of opsonins, as it appears that there is usually a zone of inhibition the effect of which is that where an abundance of opsonins is present the phagocytosis is more active in the diluted serum than in concentrated serum.

3. The use of heated serum entails the elimination of a large number of probably unspecific reactions.

4. The citrated blood is collected from the donor just prior to use, so that the phagocytic leucocytes are in full activity.

5. Only one control slide with donor's blood is required for an entire series of serum tests, whereas Huddleson advises a control without bacteria for each citrated blood specimen in order to preclude confusion with bacteria with basophil granulae in the leucocytes.

Some of the author's experiences with the above cytophagic reaction are described below.

Patients suffering from undulant fever always give a positive phagocytic reaction when the agglutination or complement-fixation test is positive.

710 specimens from patients with a positive agglutination and complement-fixation test all gave a strong positive phagocytic reaction. In most cases the phagocytosis is more marked in the 1:10 dilution than in concentrated serum (on account of the zone of inhibition).

Ten examples are given of the sero-reactions of undulant fever patients whose disease had lasted for various periods (Table 1).

Table I.

No.	Agglutination	Complementfixation	Phagocytosis with serum from the patient								Days from beginning of fever to seroreaction
			0,02 c. c.				0,002 c. c.				
1	400	400	19	5	1	0	22	1	2	0	3
2	400	800	10	12	3	0	20	4	1	0	2
3	3200	400	21	4	0	0	20	2	3	0	8
4	1600	800	19	5	1	0	23	1	1	0	17
5	1600	1600	14	11	0	0	20	5	0	0	10-
7	3200	800	16	6	3	0	21	3	1	0	32
8	3200	800	16	6	3	0	21	3	1	0	32
8	400	400	18	6	1	0	19	6	0	0	43
9	800	3200	15	8	2	0	21	4	0	0	53
10	400	400	18	7	0	0	22	3	0	0	56

The following are some examples of how the cytophagic reaction carried out with the above technique can reveal the presence of an abundance of opsonins, notwithstanding that this could not be demonstrated with Huddleson's technique (Table II).

Table II.

Serum c. c.	Phagocytosis with serum							
	Healthy person				Patient with Brucellosis			
0,02	16	5	4	0	11	12	2	0
0,006	2	3	9	11	16	9	0	0
0,002	0	0	9	16	22	3	0	0
0,0006	0	0	1	24	18	2	5	0
0,0002	0	0	0	25	11	3	6	5
0,00006	0	0	0	25	0	0	1	24
Phagocytosis with citratet blood (a. m. Huddleson)	23	2	0	0	1	15	9	0
Agglutination	50				800			
Complementfixation	0				200			
Skin test	0				+ +			

It appears that in the blood of patients with undulant fever the opsonin content is high and can be demonstrated in 0.0002 c. c. serum. The maximum phagocytosis is at 0.002 c. c., whereas at higher serum concentration it decreases. With Huddleson's technique, in which the patient's own blood is employed and consequently there is a large quantity of serum containing antibodies, the phagocytosis is markedly weak.

With Huddleson's technique there was marked phagocytosis in the healthy person, but the reaction disappeared almost immediately when the serum was titrated, and at 0.002 c. c. serum there was only a slight phagocytosis. Nor did this patient present any sign of infection, and the allergic skin test (with the Brucellergen) was negative.

When using unheated (native) serum there will always

be a fairly large number of weakly positive reactions, even in individuals with a negative skin reaction (probably owing to the presence of normal-opsonin). These weak reactions are eliminated by heating, whereas the specific reaction (owing to immune-opsonin) does not weaken, at any rate not greatly (see Table III).

Table III.

Diagnosis	Phagocytosis with serum 0,02 c. c.								Aggluti- nation	Comple- ment- fixation	Skin test
	Native				Heated 56° C.						
Healthy	0	0	13	12	0	0	0	25	—	—	—
Healthy	0	7	18	0	0	1	1	23	—	—	—
Cancer coli	7	13	5	0	3	7	15	0	25	25	++
Brucellosis	10	11	4	0	10	12	3	0	800	400	+

Two examples are shown below for the purpose of demonstrating how the author's cytophagic reaction and Huddleson's cytophagic reaction, the agglutination and complement-fixation test, result in individuals after a skin reaction with Huddleson's Brucellergen*) (see Table IV).

Table IV shows that:

Two individuals with a negative skin test (Nos. 1 and 2) form antibodies after the skin test, but only in one of them (No. 2) to any marked degree. It should be observed that, as shown in the table, the blood of this individual contained a certain but small quantity of opsonin prior to the skin test. On the 14th day the agglutination test was positive 1:50, and the cytophagic test with serum rather strongly positive.

In No. 3 (who previously had been employed tending cows) the skin test was strongly positive (60 mm). Before the test, too, the blood of this individual contained a certain quantity of opsonin, but no agglutinin or complement-fixing antibody.

*) The Brucellergen was kindly placed at my disposal by Dr. Huddleson.

Table IV.

Days after perfor- mance of skin test	Phagocytosis with serum from the patient				Phagocytosis with citrated blood (a. m. Huddleson)	Agglu- tination	Comple- ment- fixation							
	0,02 c. e.		0,002 c. c.											
No. 1 skin test negative.														
0	0	0	0	25	0	0	0	25	—	—				
7	0	0	7	18	0	0	0	25	0	2	14	9	—	—
14	0	7	16	2	0	0	0	25	0	7	16	2	—	—
21	0	0	14	11	0	0	0	25	3	14	8	0	—	—
42	0	0	8	17	0	0	0	25	0	0	12	13	—	—
77	0	0	0	25	0	0	0	25	0	0	0	25	—	—
No. 2 skin test negative.														
0	0	0	9	16	0	0	0	25	10	12	3	0	—	—
7	4	13	8	0	0	0	0	25	17	6	2	0	—	—
14	22	3	0	0	0	3	19	3	9	11	5	0	50	—
21	23	2	0	0	0	0	4	21	17	8	0	0	50	—
42	16	5	4	0	0	0	11	14	20	5	0	0	25	—
77	8	9	8	0	0	0	0	25	20	4	1	0	10	—
No. 3 skin test strong positive.														
0	11	8	6	0	0	0	0	25	20	5	0	0	—	—
7	18	7	0	0	0	0	0	25	21	4	0	0	50	—
14	21	4	0	0	22	3	0	0	16	7	2	0	400	200
21	18	7	0	0	21	4	0	0	15	8	2	0	400	400
42	14	9	2	0	21	4	0	0	7	15	3	0	800	100
77	11	12	2	0	20	3	2	0	22	3	0	0	400	100

On the 14th day after the skin test the cytophagic test with serum diluted to 1:10 was strongly positive, in addition to the agglutination and complement-fixation tests. On the 42nd day the agglutination test reached the maximum (1:800). It is worth nothing that in Huddleson's phagocytosis the opsonin quantity had apparently decreased, but on observing the phagocytosis with the serum this assumption proves to be incorrect; in fact, there is so much opsonin that the phagocytosis is more marked in the dilution than in concentrated serum.

These examples are merely extracts from a large material which as a whole confirms the observations described above.

Summary.

A description is given of a cytophagic test in Brucellosis using heated patient serum, bacteria suspension and citrated blood without opsonins (from a donor).

This test shows that serum from patients with undulant fever contains immune opsonins at a very early phase of the disease.

The reaction is more sensitive than the agglutination and complement-fixation test. It has certain advantages to the cytophagic reaction of Huddleson, as the blood does not need to be examined closely after collection, besides it reveals an inhibition of the phagocytosis, which occurs, when there is an especially high opsonin content in the blood. After a skin test (with Huddleson's Brucellergen) there occur immune opsonins, most markedly if there is already a small antibody content. If the skin test is positive there occurs an abundance of antibodies (agglutinins, complement-fixing antibodies and immune-opsonins), an expression of the fact that a previously infected organism can quickly mobilize its antibodies.

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UNTERSUCHUNGEN ÜBER DAS VORKOMMEN, DEN BAU UND DIE PATHOGENESE VON META- PLASTISCHEM PLATTENEPITHEL IN DER SCHLEIMHAUT DES CORPUS UTERI.

Von *E. Pelkonen*.

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Einleitung.

Die Metaplasiefrage ist schon seit fast einem Jahrhundert Gegenstand des Interesses der Pathologen und Kliniker gewesen. Die Auffassungen über die Metaplasie sind jedoch bedeutend auseinandergegangen. So haben gewisse Pathologen das Vorkommen der Metaplasien als blosser Einbildung betrachtet und mithin ihre Existenz ganz geleugnet. Andere haben in ihnen Gewebe gesehen, die durch embryonale Störungen oder aus Missbildungen entstanden sind (*Cohnheim*). Einige haben ihre Entstehung als ein Einwachsen oder eine Einwanderung von umgebendem Gewebe in eine fremde Umgebung erklärt, und schliesslich haben einige das Auftreten von ortsfremdem Gewebe nur als eine formale Akkomodation aufgefasst (*v. Hansemann*).

Ogleich heute Einigkeit darüber herrscht, dass es Metaplasien, allerdings in recht engen Grenzen, wirklich gibt, sind die Ansichten über das Vorkommen von Metaplasien in verschiedenen Organen, aber namentlich über ihre Genese immer noch geteilt.

Was die Entstehung der Metaplasien betrifft, ist jedoch immer allgemeiner die früher herrschende Virchowsche Lehre aufgegeben worden, dass die Bildung von heterologem Gewebe

durch die Persistenz von Zellen stattfindende. Das Zustandekommen der Metaplasien wird heute als ein verwickelter biologischer Vorgang aufgefasst, der wohl am ehesten als eine mit mancherlei Umdifferenzierungen geschehende Regeneration betrachtet werden kann. Die metaplastische Umwandlung erfolgt dabei durch die Tätigkeit von morphologisch indifferentem Zellgewebe.

Die betreffenden Zellen, die also die Eigenschaft besitzen, heterologes Epithel zu bilden, sind u. a. nach *Ribbert* auf ontogenetische Anomalien zu beziehen, was vor allem eine Art verkappter Missbildung bedeuten würde. Nach Ansicht der Anhänger der indirekten Metaplasie (*Lubarsch, Schridde, Borst* u. a.) findet dagegen in diesen Zellen, den Basalzellen, zuerst durch Teilung eine Entdifferenzierung statt, und die so gebildeten Zellen sind erst dann imstande, fremdes Epithel zu bilden. *Teutschlaender* weist diese indirekte Theorie als nutzlose Hypothese ab und betrachtet die Epithelmetaplasie als einen regenerativen, gleichgerichteten, direkt prosoplastischen Entwicklungsprozess, bei dem die physiologischen Regenerationszellen als verhältnismässig indifferente Gebilde eine ausserordentlich bedeutende Rolle spielen. *Saxén*, der eine seiner Arbeiten dem Studium des metaplastischen Plattenepithels in der Schleimhaut der Kieferhöhle und in Nasenpolypen gewidmet hat, kommt zu einem ähnlichen Ergebnis wie *Teutschlaender*. Nach ihm erklärt sich die Umwandlung des Flimmerepithels in Plattenepithel in den Nasenpolypen als eine anisogene Regeneration, »bei der die Basalzellen des Flimmerepithels direkt, ohne vorhergehende Rückdifferenzierung zu proliferieren beginnen und neues, höher entwickeltes Epithel, mit anderen Worten Plattenepithel mit allen für dasselbe typischen Eigenschaften bilden«. Die Entstehung dieses Plattenepithels führt *Saxén* in den Nasenpolypen ausser auf den bakteriell-toxischen auf einen mechanischen Reiz zurück.

Über die Bildung des dann und wann in der Schleimhaut des Corpus uteri vorkommenden Plattenepithels ist keine volle Einstimmigkeit erzielt worden. Die bei Neugeborenen und Kindern auftretende Plattenepithelbildung hält *R. Meyer* für

eine kongenitale Anomalie, bei Erwachsenen für häufig von dem Zervikalkanal her eingewandertes oder eingewachsenes Epithel. Eine eigentliche Metaplasie konnte *Meyer* nicht in der Korpusschleimhaut feststellen, dagegen fand er aber in gewissen von ihm untersuchten Fällen zwischen den basalen Zellen des Zylinderepithels liegende plasmaarme Zellen mit runden und gut färbbaren Kernen, die als indifferente Zellen, wie im Zervikalkanal, ortsungewöhnlich zu Plattenepithel prosoplasieren und von der Oberfläche her unter dem Zylinder-epithel in die Drüsen fortkriechen. *Deelman* hält es für möglich, dass das oberflächliche Epithel des Korpus aus dem Zervix oder der Portio eingewandert sein kann, aber er führt Fälle an, in denen man sich denken kann, dass auch dieses ebenso wie das in den Drüsenschläuchen auftretende Epithel durch direkte Metaplasie entstanden ist. Überhaupt stellen die in der Korpusschleimhaut zu konstatierenden Atypien, zu denen auch die Plattenepithelformationen gehören, nach *Deelman* einen wichtigen Teil der Metaplasien dar, sowohl die sog. progressive Metaplasie (Prosoplasie) wie auch die regressive (anaplastische) Metaplasie.

Über das Vorkommen des Plattenepithels in der Schleimhaut des Corpus uteri. Allgemeines.

Sehen wir die Literatur über das Vorkommen des Plattenepithels in der Schleimhaut des Corpus uteri genauer durch, so finden wir, dass der Erscheinung in zahlreichen Veröffentlichungen Beachtung geschenkt worden ist. Als erster hat *Zeller* bereits i. J. 1885 die Frage behandelt. Plattenepithel ist sowohl als oberflächliche Plattenepitheldecke wie als Plattenepithelformation in den Drüsenschläuchen des Korpus festgestellt worden. Diese Erscheinungen sehen wir mit zahlreichen Namen belegt, wie z. B. »Psoriasis«, »Ichthyosis«, »Leukoplakia uteri«, »Epidermisierung«, »Cholesteatom«, »Epithel-metaplasien«, »Plattenepithelknötchen«. In dem Schrifttum über das in der Korpusschleimhaut auftretende Plattenepithel ist jedoch nicht immer genügend zwischen Korpus- und Zer-

vixepithel unterschieden und auch nicht mit hinreichender Genauigkeit versucht worden, für die Feststellung des geschichteten Plattenepithels die diesem eigentümlichen Elemente zu analysieren. Wenn man, von diesen Mängeln abgesehen, noch die Möglichkeiten zu Irrtümern bezüglich des Karzinoms bedenkt, so lässt sich auf Grund der einzelnen veröffentlichten Fälle, von denen die meisten Curettenmaterial sind, durchaus nicht immer sicher entscheiden, wann im Korpusepithel wirklich geschichtetes Plattenepithel mit den für dasselbe erforderlichen Eigenschaften vorgelegen hat, wann es sich möglicherweise nur um Zervixepithel oder beginnendes Karzinom oder z. B. nur um eine Atypie bestimmter Art (vgl. *Deelman*) gehandelt hat und wann schliesslich bestimmte Atypien, z. B. abgeplattetes Epithel, nicht von echtem Plattenepithel mit allen zugehörigen strukturellen Besonderheiten unterschieden worden ist. Es steht jedoch ausser Zweifel, dass in gewissen Fällen in der Korpusschleimhaut geschichtetes, ja verhorntes Plattenepithel festgestellt worden ist. Über diese Fälle hat *R. Meyer* in seiner umfassenden Untersuchung gehandelt.

Plattenepithel im Korpus ist zuerst bei Neugeborenen und Kindern angetroffen worden (*v. Friedländer, R. Meyer, Hengge, Natanson, Björkenheim* u. a.). Plattenepithelinseln sind in der Schleimhaut des kindlichen Korpus nach den Untersuchungen von *Natanson* verhältnismässig häufig, aber eine grössere Ausbreitung des Plattenepithels im Gebiet der Schleimhaut gehört nach *Meyer* schon zu den Seltenheiten. Dieses Verhalten, wobei es sich also um eine von der Norm abweichende Differenzierung des Epithels handelt, betrachtet *Meyer* als eine kongenitale Anomalie.

Auch bei Erwachsenen hat man das Vorkommen von Plattenepithel in Form sogenannter Plattenepithelknötchen in hyperplastischen Korpusschleimhäuten und in Korpuspolypen konstatiert. Solcher Fälle hat *Meyer* unter 2000 von ihm untersuchten Curettenfällen im ganzen elf beobachtet. *Deelman* hält sie für viel häufiger. Ähnliche Knötchen sind auch von anderen Forschern festgestellt worden (*Hunziger, Engelhorn,*

Polano, Sitzenfrey, Aschheim, Ahlström, Schiller u. a.). *Deelman* sieht in ihnen am ehesten Metaplasien. Nach *Meyers* Ansicht deutet ihre Neigung, als eine Art polypenähnlicher »Epithelbäumchen« aufzutreten, einigermaßen auf kongenitale Anomalien. Einen Zusammenhang der Epithelknötchen mit Karzinomen haben *Meyer* und *Deelman* nie nachweisen können.

In gewissen Fällen war zu konstatieren, dass das in der Korpusschleimhaut auftretende Plattenepithel vom Zervikalkanal her eingewandert oder eingewachsen war. So sind ein von *Sitzenfrey* und ein von *Rosthorn* beschriebener Fall aufzufassen, in denen geschichtetes und verhorntes Plattenepithel im Zervikalkanal und überall in der Uterushöhle anzutreffen war.

Geschichtetes Plattenepithel ist ferner bei gewissen Krankheiten vorgekommen. So haben z. B. *Wertheim* und *Menge* es bei Gonorrhöe und *Deelman* bei Endometritiden nachgewiesen. Bei alten Individuen konnte Plattenepithel auch in Pyometrafällen festgestellt werden (*Zeller, Bondi* u. a.). Nach *Meyer* sind auch Sarkom, Lues und Tuberkulose als ätiologische Faktoren angegeben worden. Durch traumatische Ursachen ausgelöste regenerative Veränderungen haben mitunter zur Bildung von Plattenepithel geführt (*Werth, Deelman* u. a.). Und schliesslich haben einige Autoren (*Möricke, Franqué, Björkenheim* u. a.) gezeigt, dass bei alten Frauen Plattenepithel vorkommt, das aus starker Differenzierung infolge von Inaktivität des Epithels erklärt worden ist.

Das Material.

Als ich die vorliegende Untersuchung in Angriff nahm, deren Hauptzweck es war, das mengenmässige Vorkommen des Plattenepithels in der Schleimhaut des Corpus uteri zu verfolgen und, soweit möglich, die Genese dieses ortsfremden Epithels aufzuklären, war ich mir der Schwierigkeiten bewusst, die die Beschaffung geeigneten Materials verursachen würde. Aus früheren Untersuchungen war nämlich hervorgegangen, dass das Auftreten von Plattenepithel an fremdem Ort, d. h. an der Stelle von Zylinder- oder Flim-

merepithel, bei Infektionen und vor allem bei chronischen Infektionen am häufigsten war. Derartige Fälle aus der Universitäts-Frauenklinik in Helsinki, aus dem mein gesamtes Material stammt, zur Untersuchung zusammenzubringen, war nur in verhältnismässig beschränktem Masse möglich. Das beruhte darauf, dass in der genannten Klinik danach gestrebt worden ist, alle operativen Eingriffe — sowohl Abrasion als Laparotomie — bei entzündlichen Krankheiten sorgfältig zu vermeiden. Eine andere Schwierigkeit hat — wie anderswo — die Unterscheidung der Korpusschleimhaut im Curettenmaterial von den aus dem Zervix oder der Portio herrührenden Gewebsstücken bereitet.

Die Einsammlung des Materials habe ich während zwei Jahren (1938—1939) ausgeführt. In dieser Zeit habe ich das ganze in unserer Klinik zusammengekommene Korpusschleimhautmaterial durchgesehen, das sowohl die Curettenproben als die bei den Operationen aus der Korpusschleimhaut entnommenen Proben umfasst. Die Zahl der Fälle war im ganzen 1000, und von diesen waren über drei Viertel curettiert. Somit sind alle die Fälle näher untersucht worden, in denen die Atypie in Form einer an Plattenepithel erinnernden oder deutlichen Plattenepithelformation auftrat oder wahrscheinlich an die Schleimhaut des Korpus grenzte. Solcher Fälle hat das ganze Material über 30 enthalten.

Meine erste Aufgabe bei der Untersuchung des so analysierten Materials war, die Fälle zu bestimmen, in denen die Plattenepithelformationen sicher in der Korpusschleimhaut selbst auftraten und in denen die Bildung dieser Schleimhautatypien aller Wahrscheinlichkeit nach örtlicher Natur war und nicht z. B. vom Zervikalkanal oder von der Portio her stattfand. Dies ist meines Erachtens möglich geworden durch Beschränkung auf die Fälle, in denen sich die jeweils feststellbare Plattenepithelformation organisch und unstreitig an die sie umgebende deutlich erkennbare Korpusschleimhaut anschloss und ausser derartigen Herden gleichzeitig Epithelgebiete in einem unvollständigeren Differenzierungsstadium zu konstatieren waren. Die Unterscheidung der Korpusschleimhaut von der Schleimhaut des Zervix habe ich noch durch Muzinfärbung zur Feststellung von Zervixepithel und besonders der darin befindlichen Schleimdrüsen sichergestellt. Die auf diese Weise ausgeführte Analyse des Materials, bei der von mehreren Proben Serienschnitte angefertigt wurden, hat nur vier die obenerwähnten Bedingungen erfüllende Fälle ergeben.

Die Fixierung des zur Untersuchung bestimmten Materials erfolgte in 10 % Formalin oder Alkohol. Die Schleimhautproben wurden dann auf gewöhnliche Weise in Paraffin eingebettet. Zur allgemeinen Färbung wurde Delafields Hämatoxylin und zur Nachfär-

bung Eosin und van Giesons Lösung benutzt. Bei der Differenzierung der Exsudatzellen wurde zu der kombinierten May-Grünwald-Giemsa-Methode gegriffen. Für die Untersuchung des Keratins kam zur Anwendung Gram- sowie Indigorot-Färbung, zur Darstellung der Plasmafibrillen und der Interzellularbrücken Heidenhains Eisenalaunhämatoxylin-Färbung und zum Nachweis von elastischen Fasern im Bindegewebe Weigerts Elastinfärbung.

Wie erwähnt, habe ich mein ganzes Material in der Universitätsfrauenklinik zu Helsinki gesammelt, aber die Untersuchung selbst habe ich sowohl in dieser Klinik als im Pathologisch-anatomischen Institut der Universität Helsinki ausgeführt. Es sei mir gestattet, an dieser Stelle Herrn Prof. *Arno Saxén* nicht nur für das Thema meiner Untersuchung, sondern vor allem für die Anleitung bei der Arbeit und für die Mühwaltung, die die Durchsicht und Prüfung der zahlreichen Präparate erfordert hat, meinen besten Dank auszusprechen.

Auf Grund der oben beschriebenen Analyse habe ich in meinem ganzen 1000 Fälle umfassenden Material nur in vier Fällen das Vorkommen von Plattenepithelformationen in der Schleimhaut des Corpus uteri festgestellt. Im folgenden gebe ich die über diese Fälle erhobenen Befunde wieder.

Fall 1.

65jährige Arbeiterwitwe. J.-Nr. 605/III 1937 und J.-Nr. 160/III 1938. Auf der Abteilung 4.—15. 12. 1937 und 23.—29. 3. 1938. Diagnose: Pyometra.

Menarche mit 15 J. Menopause mit 57 J. Die Menses waren regelmässig. P. 21—28 T.; D. 5—6 T. Seit 1899 verheiratet, 5 Kinder, das jüngste 20 J. Partus normal. Fehlgeburt 1906. Keine Abrasion. Ein halbes Jahr vor der Aufnahme in die Klinik begann ein spärlicher blutiger Fluss, der in einigen Tagen braun-grün-gelb wurde und so bis zur Ankunft in der Klinik fort dauerte. Der Fluss hat nach der Pat. keinen übleren Geruch gehabt. Keine Schmerzen. Kein Fieber.

Allgemeinzustand bei der Aufnahme gut. Hb 72 (Sahli). SR 9/25 mm. WR —, Harn = 0.

Vulva und Vagina senil-atrophisch. Portio atrophisch, an der Oberfläche rauh. C. ut. klein in Retroversion, nicht ganz frei beweglich. Die Adnexe sind nicht im einzelnen palpabel, an ihrer Stelle keine Resistenz und keine Empfindlichkeit. Parametrien weich, nicht schmerzhaft.

Abrasion I 7. 12. 1937. Bei der Dilatation reichlich eitrig-Fluss. Die Wand des C. ut. fühlt sich glatt an. Man bekommt spärlich

Korpusschleimhaut, wovon die unten beschriebene Probe a) herührt.

Nach der Entlassung aus der Klinik hat der blutige und eitrige Fluss fortgedauert.

Abrasion II 24. 3. 1938. Bei der Dilatation reichlich eitriger Fluss. Die Wand des C. ut. fühlt sich glatt an, von ihrer spärlichen Schleimhaut stammt die Probe b), die unten beschrieben ist.

Mikroskopischer Befund: Abrasion I, Probe a). Korpusschleimhaut verhältnismässig dünn, im Stroma durchgehend eine ausserordentlich starke Entzündungszelleninfiltration, in der die Leukozyten dominieren, die aber auch reichlich Lymphozyten sowie Plasmazellen enthält. Die Stromazellen sind spindelförmig und ziemlich spärlich. Drüsenschläuche mässig reichlich. Schläuche klein, ihr Epithel von zylinderförmigen und kubischen Zellen gebildet, im allgemeinen normal. An einigen Stellen sieht man jedoch Drüsenschläuche, deren Epithel aus mehreren Schichten von kubischen Zellen besteht. An gewissen Stellen hat das Epithel der Schläuche das Aussehen von Übergangsepithel angenommen, dessen Zellen bedeutend grösser als die in der Probe auftretenden normalen Epithelzellen, an einzelnen Stellen sogar 5—6 mal so gross sind und wechselnd längliche, kubische, polygonale und andere Form zeigen. Diese Zellen sind im allgemeinen plasmareich. Die kleineren und am meisten an die gewöhnlichen Epithelzellen der Schläuche erinnernden Zellen liegen meistens basal und haben grosse und relativ gut färbbare Kerne. Die Kerne der grösseren, oft näher bei dem Zentrum des Schlauches liegenden Zellen sind gross, aber im allgemeinen schwächer färbbar, an gewissen Stellen pyknotisch, und es sind sogar nicht in allen Zellen Kerne zu konstatieren. Einige von diesen Zellen sind vakuolisiert, ja in gewissen umfassen die Vakuolen beinahe die ganze Zelle (Abb. 1). Die Interzellularräume sind schmal, und Interzellularbrücken sind an gewissen Stellen, wiewohl schwach ausgebildet, festzustellen. Im Deckepithel ist keine Umwandlung der vorstehend beschriebenen Art in der Probe zu sehen, sondern es macht den Eindruck eines ziemlich regelmässig einreihigen, von kubischen Zellen gebildeten Epithels, auf dessen Aussenfläche ein zusammenhängender verbindender Saum zu bemerken ist.

Abrasion II, Probe b). Schleimhaut des Korpus verhältnismässig dünn. Stromazellen spindelförmig, wenig zahlreich und wegen der ausserordentlich reichlichen Entzündungszellen schwächer sichtbar. Von den Entzündungszellen sind die Leukozyten am stärksten repräsentiert, aber auch Lymphozyten und Plasmazellen sind überall zahlreich zu konstatieren. Die Drüsenschläuche sind im allgemeinen mässig klein und an Zahl gering sowie an manchen Stellen normal

und mit einem einreihigen kubischen Epithel versehen. Hier und da findet man jedoch im Epithel der Schläuche ebenso wie an einigen Stellen im Deckepithel eine an Übergangsepithel erinnernde Umwandlung (Abb. 2) von der Art, wie sie in der vorhergehenden Probe beschrieben wurde. Anderswo ist eine Umwandlung nur in

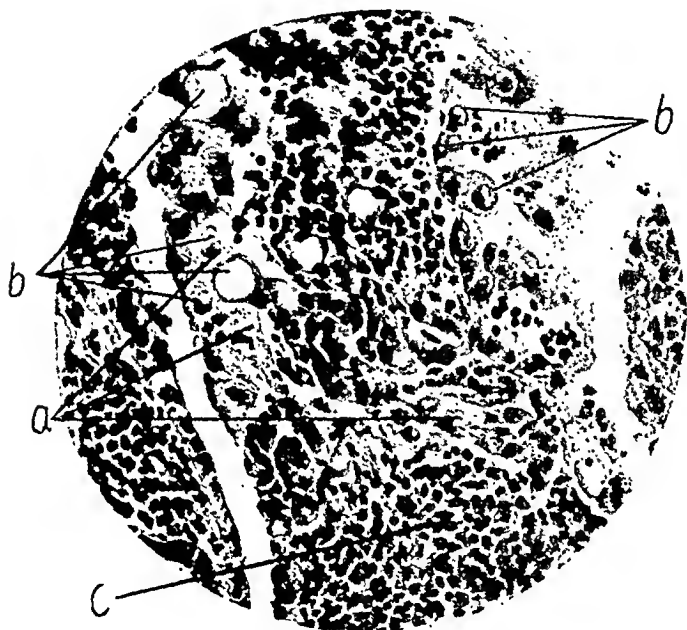


Abbildung 1.

Frühes Differenzierungsstadium. a) Grosse Epithelzellen eines Drüsenschlauches, die an Übergangsepithel erinnern, b) vakuolierte Epithelzellen, in denen die Kerne teils pyknotisch, teils ganz verschwunden sind, c) Entzündungszellen.

einem Teil der Drüsenschlauchwand festzustellen. Das basale Zellengewebe ist in diesen alterierten Partien meist kubisch. Es wird von dem Stromagewebe an manchen Stellen durch eine an eine Membrana propria erinnernde Schicht getrennt, meist aber schliesst es sich unmittelbar an das Stroma an. Diese basalen Zellen treten bald einreihig, bald mehrreihig oder bisweilen völlig unregelmässig aneinandergesetzt auf. Ihre Kerne sind im allgemeinen gross und gut färbbar. Die diese basalen Zellen bedeckenden Zellen sind grösser und von Form stärker wechselnd, die polygonale und langzylindrische Form ist bei ihnen jedoch am häufigsten. Die Kerne sind

schwächer färbbar, und die Kernalterationen werden im allgemeinen häufiger, je mehr man sich von der basalen Schicht entfernt. Die Interzellularräume variieren in bezug auf die Grösse, an manchen Stellen sind Entzündungszellen in sie eingedrungen. Die Interzellularbrücken sind stellenweise schwach, aber schon deutlich zu

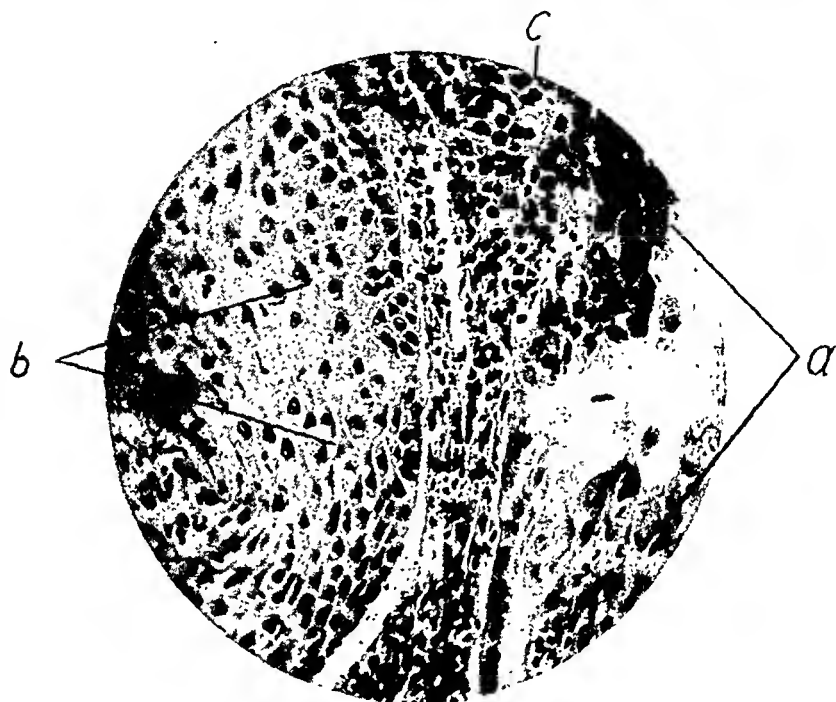


Abbildung 2.

Differenzierungsstadien verschiedenen Grades. a) Frühe, an Übergangsepithel erinnernde Entwicklungsstufe, b) weiter differenziertes Epithel, das schon den Bau von Plattenepithel mit Basal- und Stachelzellen und Interzellularbrücken zeigt, c) Entzündungszellen.

erkennen. Hier und da kommen die oben beschriebenen basalen Zellen gar nicht vor, sondern diese grösseren polygonalen Zellen grenzen unmittelbar in Form eines ein- oder mehrschichtigen Gewebes an das Stroma.

An einigen Stellen ist die Differenzierung des Epithels weiter fortgeschritten. So bemerken wir in der Probe gewisse Partien, wo das Epithel schon ziemlich vollständig den Bau von geschichtetem Plattenepithel zeigt (Abb. 2 und 3). Diese Epithelformation hebt sich an einigen Stellen von dem Stromagewebe mittels eines dichten

teren, einer Membrana propria ähnlichen Gewebes ab, das jedoch meistens ganz fehlt. Das Basalzellengewebe ist an wenigen Stellen normal, von bipolaren oder kubischen und senkrecht zu ihrer Unterlage orientierten Zellen gebildet. Meistens besteht es aus in mehreren Reihen oder völlig unregelmässig liegenden polymorphen, polygonalen oder langzylindrischen Zellen. Stellenweise wird das basale Zellgewebe gänzlich vermisst, und an das Stromagewebe grenzt unmittelbar eine Stachelzellenschicht. Die Dicke dieser letzteren schwankt erheblich, zwischen 2 und 10 Zellreihen. Die am basalen liegenden Stachelzellen sind an den meisten Stellen bipolar, die folgenden gewöhnlicher polygonal und die der Oberfläche am nächsten liegenden abgeplattet. Die Form der Stachelzellen kann indessen stark wechseln, und zwar auch bei den in ein und derselben Schicht liegenden, ebenso erscheinen die Schichten auch oft ganz regellos. Die Kerne der basal liegenden Zellen sind gut färbbar, dagegen färben sich die Kerne der mehr in der Mitte und vor allem der oberflächlicher liegenden Zellen schlechter; leichter oder stärker alterierte Kerne finden sich auch in den letzterwähnten Schichten zahlreich.

Die Interzellularräume in dem basaler liegenden Zellgewebe sind verhältnismässig breit und enthalten zahlreiche Exsudatzellen. Die interzellulären Fibrillen treten in dem basal gelegenen Zellgewebe deutlich, oft mit Bizzorzeroschen Knoten versehen hervor, werden aber undeutlicher in dem oberflächlicher liegenden Stachelzellengewebe, in dem auch die Interzellularräume schon schmaler und die Kerne stärker alteriert sind. Auch die Plasmafibrillen sind in den basalen Schichten am besten zu sehen. Wo das Basalzellengewebe am regelmässigsten ist, verlaufen die Fibrillen in der Längsrichtung der Zellen, im Stachelzellengewebe sind sie teilweise tangential zur Oberfläche angeordnet, treten aber doch meist völlig regellos auf, wie es der regellose Bau des Zellgewebes voraussetzt.

Eine Keratohyalinschicht und ein Stratum lucidum fehlen in den beschriebenen Epithelformationen. Die obersten Zellschichten sind nur von abgeplatteten Zellen gebildet, deren Kerne schlechter färbbar, stellenweise nur als Kernschatten sichtbar oder ganz verschwunden sind. Eine deutliche Verhornung ist nicht zu konstatieren.

In Probe b) kann man das Verhältnis des Drüscnepithels zu der Plattenepithelformation verfolgen. An gewissen Stellen bemerkt man Übergangspartien, in denen ein ziemlich normales Epithel und ein schon in Übergangsepithel umgewandeltes Epithel sich vereinigen oder zu Plattenepithel werden. Von den Plattenepithelinseln umfasst eine wahrscheinlich Deckepithel. Die mancherlei in dem Curettenmaterial wirksamen mechanischen Faktoren erschweren na-

türlicherweise eine klare Darstellung der genannten Übergangspartien. In der rechten Hälfte von Abbildung 3 konstatieren wir jedoch einen Epithelzug, in dem die Zellen atypisch, regellos angeordnet sind und in dessen Zellen wir regressive Veränderungen, Vakuolation, schwächere Färbbarkeit der Kerne, beginnende Kernpyknose

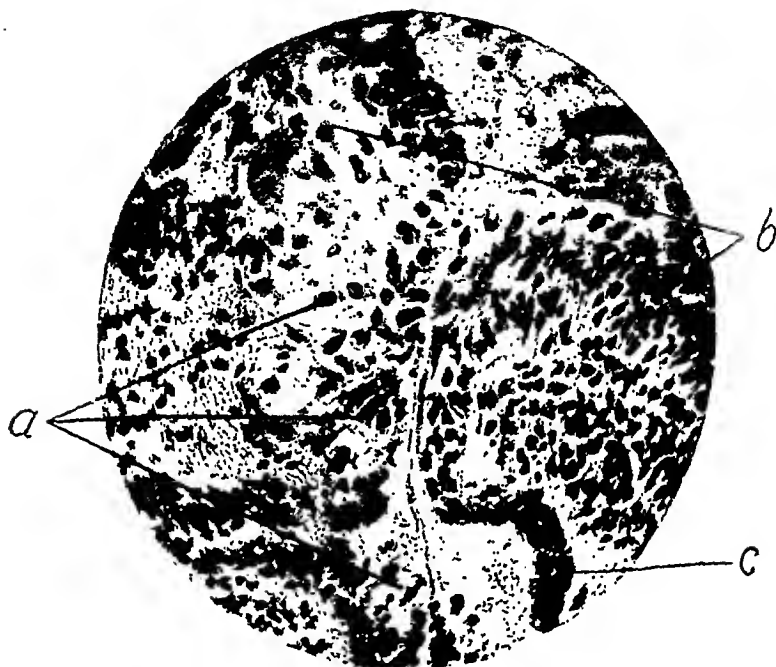


Abbildung 3.

Differenzierungsstadien verschiedenen Grades. a) Frühere Entwicklungsstufe, b) weiter differenziertes Epithel, das schon primitives Plattenepithel mit unregelmässigen Basal- und Stachelzellen und schwach ausgebildeten Interzellularbrücken vertritt, c) eine Epithelzellenreihe, in der die regressiven Veränderungen der Zellen zunehmen, während sich die Reihe an differenziertes Epithel anschliesst.

usw. erkennen. Diese Epithelkette verschmilzt, während die Zellen sich allmählich vermehren und den Basalzellen in der nebenanliegenden Plattenepithelformation ähnlich werden, mit dem von den Basal- und Stachelzellen gebildeten Plattenepithel. In der linken Hälfte derselben Abbildung sehen wir der erwähnten Plattenepithelinsel wahrscheinlich nur angelagert eine andere Plattenepithelformation. Deren sich verschmälernder unterer Teil mit einigen losgerissenen Zellen vertritt ein weniger differenziertes Epithel, Über-

gangsepithel, dessen Vorkommen in dieser Probe früher beschrieben worden ist. In Abbildung 2 finden wir links eine differenzierte Plattenepithelinsel (die Fortsetzung der in Abbildung 3 auftretenden Insel rechts) sowie rechts ein weniger differenziertes Übergangsepithel. Obgleich die erwähnten, in verschiedenem Grade differenzierten Epithelzellengewebe einander nicht unmittelbar fortsetzen, stehen sie doch nur durch ein relativ schmales, von Exsudatzellen erfülltes und nach unten schmaler werdendes Stroma in unmittelbarer Verbindung miteinander. In den Übergangspartien beobachten wir eine Proliferation von Epithelzellen und eine Vereinigung dieser proliferierten Epithelzellen mit einem von Basalzellen oder unfertigen polygonalen Stachelzellen gebildeten Gewebe. In den letzterwähnten Zellen sowie in dem basalen Zellgewebe des bereits differenzierten Plattenepithels sind, wiewohl nicht sehr zahlreich, auch Mitosen festzustellen. Das an manchen Stellen in der Probe b) auftretende Übergangsepithel ist mithin als eine unvollständiger differenzierte Zwischenstufe des Plattenepithels zu betrachten, was auch bei der Untersuchung des ganzen Präparates sehr deutlich ersichtlich wird. Es ist offenbar, dass auf dieselbe Weise das schon in der Probe a) vorhandene Übergangsepithel als eine Übergangsstufe zu Plattenepithel aufzufassen ist, das jedoch in dieser Probe nicht nachzuweisen war.

Fall 2.

61jährige Arbeiterfrau. J.-Nr. 46/III 1938. Auf der Abteilung 7. 1.—5. 7. 1938. Diagnose: Pyometra.

Menarche mit 18 J. Menopause mit 57 J. Die Menses waren regelmässig. P. 28 T.; D. 7 T. Seit 1901 verheiratet, 11 Kinder, das jüngste 20 J. Partus normal. Keine Aborte und keine Abrasionen. — Im April 1937 begann ein bräunlicher Fluss, der vom September an deutlich blutig und stinkend wurde. Ein Jahr lang Schmerzen im Kreuz, welche nachliessen, als der Fluss reichlicher geworden war.

Allgemeinzustand bei der Aufnahme mässig gut. Hb 79 (Sahli), SR 17/35 mm. WR —, Harn = 0.

Vulva und Vagina senil-atrophisch, Portio ganz atrophisch. Eitriger Fluss aus dem Uterus. C. ut. von gewöhnlicher Grösse, weich, in Anteversio-Flexio, frei beweglich, nicht schmerzhaft. Die Adnexe sind nicht zu palpieren und nicht empfindlich. Parametrien weich, nicht schmerzhaft.

Abrasion 8. 1. 1938. Wand des C. ut. glatt. Mit dem stinkenden Eiter bekommt man spärlich Korpusschleimhaut, wovon die unten beschriebene Probe.

Mikroskopischer Befund: Korpusschleimhaut dünn, Stromazellengewebe spindelförmig, zellendicht. Stroma durchgehend von zahl-

reichen Exsudatzellen infiltriert. Die Infiltration besteht fast ausschliesslich aus Leukozyten, aber zu einem kleinen Teil auch aus Lymphozyten und Plasmazellen. Die Drüenschläuche sind klein und treten recht spärlich auf. Das Epithel der Schläuche ist ziemlich normales, von kubischen oder zylindrischen Zellen gebildetes

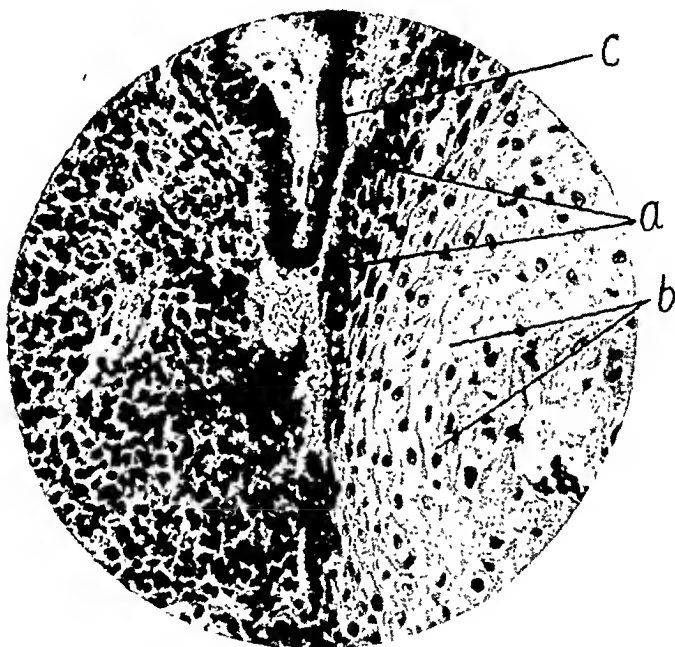


Abbildung 4.

Plattenepithelinsel. a) Basalzellen, b) Stachelzellen mit Interzellularbrücken, c) ziemlich regelmässiger Drüenschlauch der Schleimhaut.

Gewebe. Hier und da weist das Epithel jedoch eine Atypie auf: die Zellen sind gross und liegen in mehreren Schichten, oft ganz regellos. Eigentliches Übergangsepithel ist nicht deutlich festzustellen. In der Probe sieht man zahlreiche Plattenepithelinseln, und das sie umgebende Bindegewebe enthält deutliche kleine Drüenschläuche mit den Eigenschaften der Korpusschleimhaut (Abb. 4). Diese Plattenepithelinseln gehen von dem Epithel der Drüenschläuche aus oder erscheinen als Deckepithel. Die meisten sind von derselben Art wie in Fall 1. Nach dem Bindegewebe hin werden sie von einem an eine Membrana propria erinnernden Gewebe begrenzt, aber Bindegewebsspapillen sind nicht zu konstatieren. Die Anordnung der Basalzellen und Stachelzellen ist meist regellos, eine Keratohyalinschicht und ein Stratum lucidum fehlen, die Färbbarkeit der Kerne

nimmt weiter von der Basalschicht entfernt ab, im gleichen Verhältnis werden die Plasmafibrillen und die interzellulären Fibrillen dünner und undeutlicher. An gewissen Stellen ist die Epidermisierung weiter fortgeschritten. In Abbildung 5, die eine in Verbindung mit Drüsen entwickelte, von Bindegewebe umgebene Epithelinsel

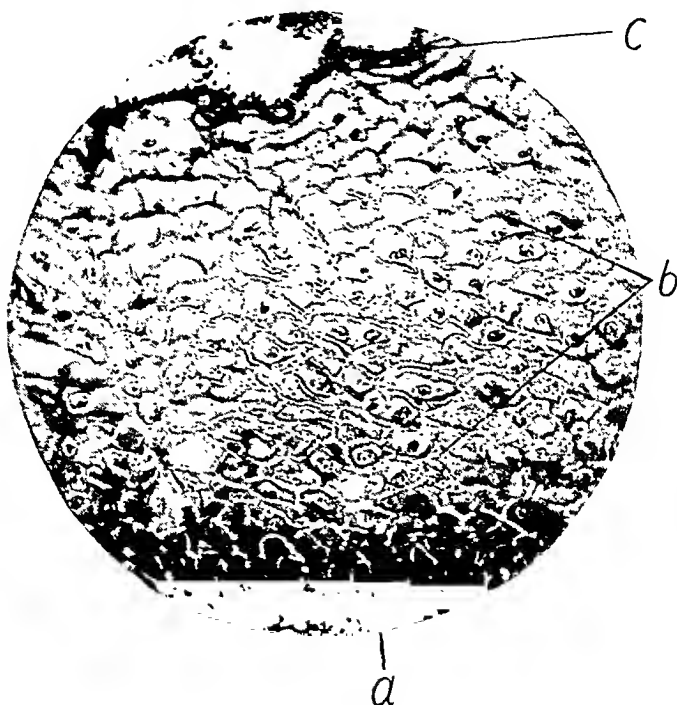


Abbildung 5.

Plattenepithelinsel. a) Basalzellen, b) Stachelzellen mit Interzellularbrücken, c) keratohyaline Schicht.

darstellt, finden wir schon eine schwach hervortretende Keratinisierung sowie eine beginnende Papillenbildung. In der das Deckepithel veranschaulichenden Abbildung 6 ist die Epidermisierung schon ziemlich vollständig. Da konstatieren wir bereits eine Bildung deutlicher Papillen. Das Basalzellengewebe ist von länglichen, regelmässigen, senkrecht zur Unterlage orientierten Zellen gebildet, die Stachelzellen sind in den tieferen Schichten polygonal, in den oberflächlicheren langzylindrisch, stellenweise breit spindelförmig und in 4—10 Zellreihen angeordnet. Die Keratohyalinschicht tritt deutlich, 2—3 Zellreihen dick hervor und ist von einer deutlich fest-

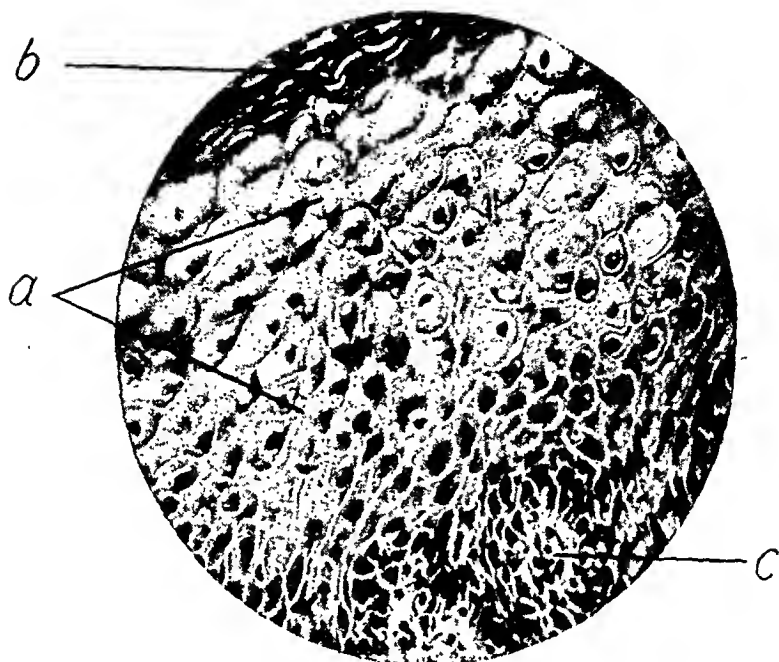
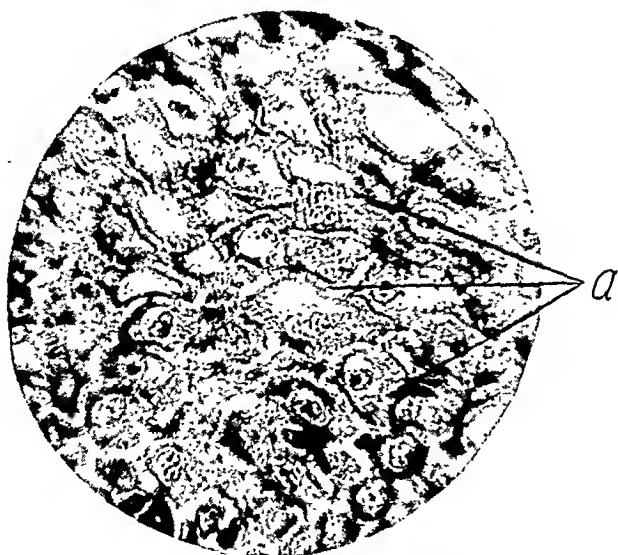


Abbildung 6.

Ziemlich vollständig differenzierte Plattenepithelinself. a) Stachelzellen mit Interzellularbrücken, b) keratohyaline Schicht mit Verhornung, c) vollständig ausgebildete Bindegewebspapille.



stellbaren Hornschicht bedeckt. Die Kerne sind in den mehr basal liegenden Zellreihen gut färbbar, in den mittleren und besonders in den oberflächlichen schwächer färbbar, ja stellenweise fehlen sie ganz. In den oberflächlichen Zellen ist auch hier und da eine Vakuolisierung zu bemerken. Die Plasma- und die interzellulären Fibrillen (Abb. 6 und 7) treten in den basalen Zellen ausserordentlich deutlich hervor, wobei sie nach der Oberfläche hin dünner und undeutlicher werden. Die Interzellularräume sind verhältnismässig breit, und man findet, dass zwischen sie sowie auch in die keratohyaline Schicht Exsudatzellen eindringen. Mitosen wurden nicht in den Präparaten nachgewiesen.

Fall 3.

37jährige Kaufmannsfrau. J.-Nr. 737/I 1938. Auf der Abteilung 7.—12. 11. 1938. Diagnose: Metropathia.

Menarche mit 15 J. Menses früher regelmässig, reichlich, schmerzlos. P. 28 T.; D. 3—4 T. Letzte regelmässige Menses 19. 6. 1938. Anhaltender 6wöchiger Fluss begann am 17. 7. 1938. Derselbe war zeitweise ausserordentlich reichlich, mitunter spärlicher, ja einige Tage fast verschwunden. Nach 8—10tägiger Pause fing ein ähnlicher, 3 Wochen dauernder Fluss an, der sich nach einer 10tägigen Pause bis zum 28. 10. 1938 fortsetzte. Vom Juli an Schmerzen im Unterleib, besonders links. Während des Augusts und Septembers einigemal Fieber bis 39°, das einige Tage dauerte. Verheiratet 1920—27 und seit 1936. Keine Partus, keine Aborte und keine Abrasionen. Früher gesund.

Allgemeinzustand bei der Aufnahme in die Klinik mässig gut, Hb 66 (Sahli), SR 9/23 mm, WR —. Im Harn reichlich Kolibazillen und Leukozyten sowie Epithelzellen. Unbedeutende Druckempfindlichkeit in der Mitte des Unterleibs.

Vulva gewöhnlich, Vagina eng; Portio virginell, C. ut. ziemlich klein, fest in Anteversio-Flexio, retroponiert, etwas nach hinten fixiert. Linke Adnexe von normaler Grösse, nicht empfindlich. Rechte Adnexe nicht genau zu palpieren, an ihrer Stelle keine Resistenz und keine Empfindlichkeit.

Abrasion 8. 11. 1938. Wand des C. ut. glatt. Man bekommt ziemlich spärlich grauliche Schleimhaut, von der das unten beschriebene mikroskopische Präparat stammt.

Mikroskopischer Befund: Korpusschleimhaut in Proliferation begriffen, verhältnismässig dick, Stroma zellenreich und von mässig zahlreichen Exsudatzellen, Lymphozyten und Plasmazellen, aber teilweise auch Leukozyten infiltriert. Die Drüsenschläuche sind von mittlerer Weite und relativ reichlich vorhanden. Reichlich Invaginationen. Schlauchepithel ziemlich normal, zellenreich. An einigen

Stellen verhältnismässig wenig ausgebildetes Übergangsepithel, dessen basale Zellen hauptsächlich polygonal sind und regellos liegen. In den oberflächlicheren Zellen zeigt sich eine ausserordentlich ausgiebige Alteration, die Kerne fehlen in denselben oft schon ganz. In dem basalen Zellgewebe tritt hier und da eine Mitose auf.

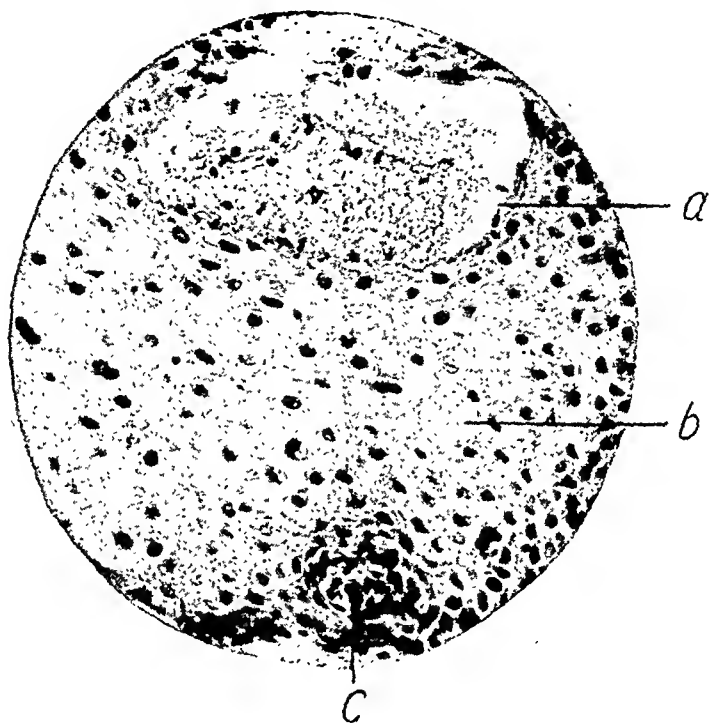


Abbildung 8.

Plattenepithelinsel. a) Beginnende Verhornung, b) Stachelzellenschicht, c) beginnende Papillenbildung.

Interzellularräume unregelmässig, im allgemeinen gross, in denselben und besonders in den oberflächlicheren Teilen reichlich Exsudatzellen. Ebenso ist das Exsudatzellengewebe in der Umgebung dieser alterierten Epithelinseln reichlicher. Interzellularbrücken sind nicht deutlich festzustellen, ebensowenig Plasmafibrillen. In den Proben beobachtet man nur einige Plattenepithelinseln im Bindegewebe (Abb. 8). Der Bau derselben erinnert an den der Herde im vorhergehenden Fall. Eine beginnende Bildung von Papillen ist schon zu konstatieren. Die Basalzellenreihen sind regellos, mitunter fehlen sie ganz. In der Stachelzellenschicht, die von 3–6 regellosen Zellreihen gebildet wird, findet man sowohl inter- als intrazelluläre

Fibrillen. Im Zentrum der Inseln tritt schon eine deutlich erkennbare Verhornung auf.

Fall 4.

25jährige Dienstmagd. J.-Nr. 608/I 1938 und J.-Nr. 104/I 1939. Auf der Abteilung 16. 9.—21. 12. 1938 und 31. 1.—13. 2. 1939. Diagnose: Salpingo-oophoritis bil. chr. Hypertrophia et metaplasia mucosae uteri. Pyelitis.

Menarche mit 16 J. Menses früher regelmässig. P. 21 T.; D. 4 T. Fluss ziemlich spärlich, Schmerzen unbedeutend. Während der zwei letzten Jahre Menses unregelmässig, dann und wann 2—3 Monate aussetzend. Fluss weiterhin ziemlich spärlich, Dauer 2—6 Tage, dabei 1—2tägige Pausen. Letzte Menses am 8.—9. 9. 1938. Keine Kinder, keine Aborte und keine Curettagen. Früher gesund. Vor $\frac{1}{2}$ Jahre 5 Tage lang Fieberperioden bis 39° (Pyelitis?). Bei den zwei letzten Menstruationen während ein paar Tagen unbedeutende Temperatursteigerung. Während 2 Jahren dann und wann leichte Schmerzen im Unterleib.

Allgemeinzustand bei der Aufnahme gut, Hb 70 (Sahli). SR 22/47 mm, Gonoreaktion (Kristensen) —, WR —, Gesamtumsatz 6 %, im Harn reichlich Staphylokokken und spärlich Erythro- und Leukozyten sowie Epithelzellen. Während der vier ersten Tage unbedeutende Temperatursteigerung bis $37,6^{\circ}$ (Neosalvarsan).

Vulva und Vagina hypoplastisch. Portio virginell. C. ut. klein, daumenspitzen gross in Anteversio-Flexio, Zervikalteil nach hinten gezogen, Fundus frei beweglich. Beide Ovarien klein, fest, fixiert, mässig empfindlich.

Während des Anstaltsaufenthalts 18. 9.—22. 11. 1939 spärlicher, ununterbrochener, mit Pausen von nur einigen Tagen auftretender blutiger Fluss aus dem Uterus. *Abrasion* I 22. 11. 1939. Wand des C. ut. glatt, man bekommt von der Korpuswand ziemlich reichlich dicke Schleimhaut. Auf das aus dieser hergestellte mikroskopische Präparat bezieht sich die untenstehende Beschreibung der Probe a).

Hiernach hörte der Fluss auf, folgende Menstruation am 21.—24. 1. 1939 spärlich und schmerzlos. *Abrasion* II 8. 2. 1939. Man bekommt reichlich dicke Korpus Schleimhaut, Probe b).

Mikroskopischer Befund: Abrasion I, Probe a). Die Schleimhaut befindet sich in der beginnenden Sekretionsphase und ist überall dick, das Stromazellengewebe ist verhältnismässig dicht und von länglichen, spindelförmigen Zellen gebildet. Eine Entzündungszelleninfiltration ist überall, an gewissen Stellen sogar mässig reichlich zu konstatieren und sie besteht im allgemeinen aus Lymphozyten und Plasmazellen, aber in den Drüenschläuchen und überhaupt an Stellen, wo die Epithelveränderungen am weitesten ent-

wickelt sind, findet man fast ausschliesslich Leukozyten. Drüsen-
schläuche treten überall reichlich, ja stellenweise aneinanderge-
drückt auf. Ihre Grösse variiert bedeutend von kleinen und mittel-
grossen bis zu ausgedehnten Höhlen, am zahlreichsten sind jedoch
die mittelgrossen Schläuche. Nur an wenigen Stellen haben die

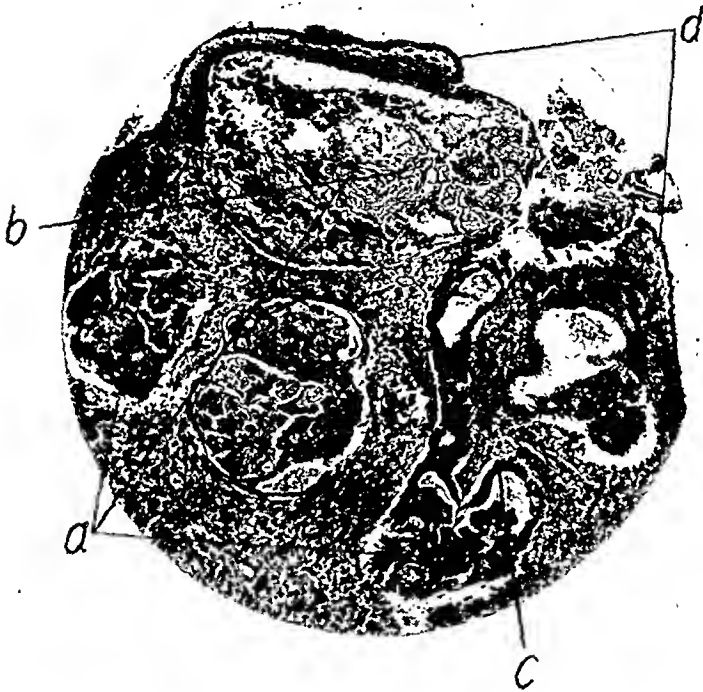


Abbildung 9.

Übersichtsbild einer Schleimhaut, in deren sämtlichen Drüsen-
schläuchen verändertes Epithel festzustellen ist. a) In verschiedenem
Grade differenziertes Epithel, b) in Nekrose übergegangenes Epithel,

- c) ziemlich wohlerhaltenes Epithel eines Drüschlauchs,
d) Deckepithel.

Schläuche sich regelmässig, mit normalen Epithelzellenreihen er-
halten, meist findet man nämlich in einem beträchtlichen Teil von
ihnen, hier und da in ziemlich allen Drüenschläuchen, Veränderun-
gen verschiedenen Umfangs im Bau des Epithels (Abb. 9). Stellen-
weise bemerken wir, dass die Veränderungen nur einen kleinen Teil
der Schleimhaut einer Drüse umfassen, während der grösste Teil
ziemlich normal geblieben ist, an anderen Stellen ist das Epithel

des ganzen Drüsenschlauches, ja das Epithelgewebe mehrerer sich aneinander anschliessender Schläuche alteriert. Das Deckepithel ist im allgemeinen normal erhalten, hier und da stellen wir jedoch auch in ihm eine Umwandlung fest.

In der Umwandlung des Drüsenschlauchepithels beobachten wir

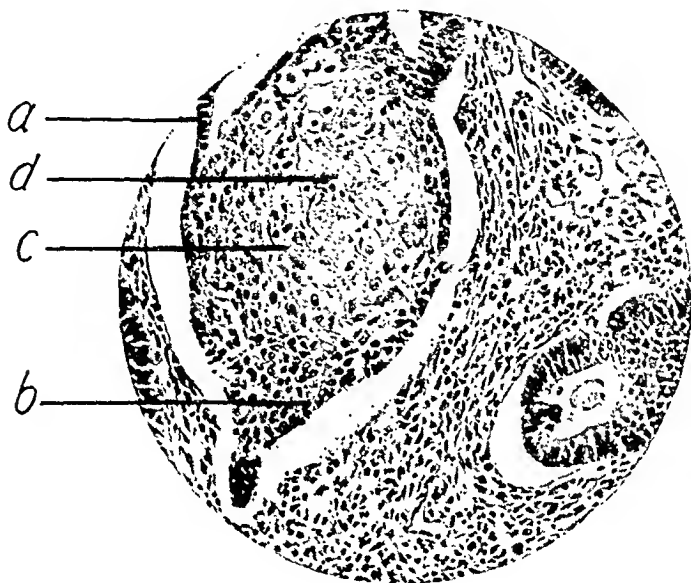


Abbildung 40.

Differenzierung des Drüsenschlauchepithels. a) Ziemlich wohlerhaltenes Epithel eines Schlauches, b) Proliferation von Epithelzellen, c) primitive Stachelzellen, d) beginnende körnige Keratohyalinisierung.

verschiedene Entwicklungsstadien. Als erstes finden wir eine bedeutende Proliferation der Epithelzellen (Abb. 10). Anfangs behalten diese proliferierten Epithelzellen die dem Zylinderepithel eigentümliche Form bei, aber verhältnismässig bald, oft schon nach der Bildung einiger Zellreihen, werden die Zellen grösser, polygonal, den Basalzellen ähnlich, während die Kerne doch noch ihre Grösse, Form und Färbbarkeit bewahren. Interzellularbrücken sind in den verhältnismässig breiten Interzellularräumen auch schon an manchen Stellen, wiewohl schwach, zu erkennen. Hier und da schliesst sich dieses Zellgewebe an Epithel an, in dem die Differenzierung weiter fortgeschritten ist. Die Zellen werden alsdann rundlich oder länglich, den Stachelzellen ähnlich, aber die Interzellularräume und -brücken werden immer undeutlicher und sind nur spärlich zu fin-

den. Die Kerne sind oft kleiner und schwächer färbbar. Dieses ganze Zellgewebe stellt keine regelmässig differenzierte Stachelzellenschicht dar, sondern ein Gewebe, dessen Zellen wohl am ehesten als abortive, in unabgeschlossener Entwicklung begriffene Stachelzellen zu betrachten sind. Manche dieser Zellen, gewöhnlich die in

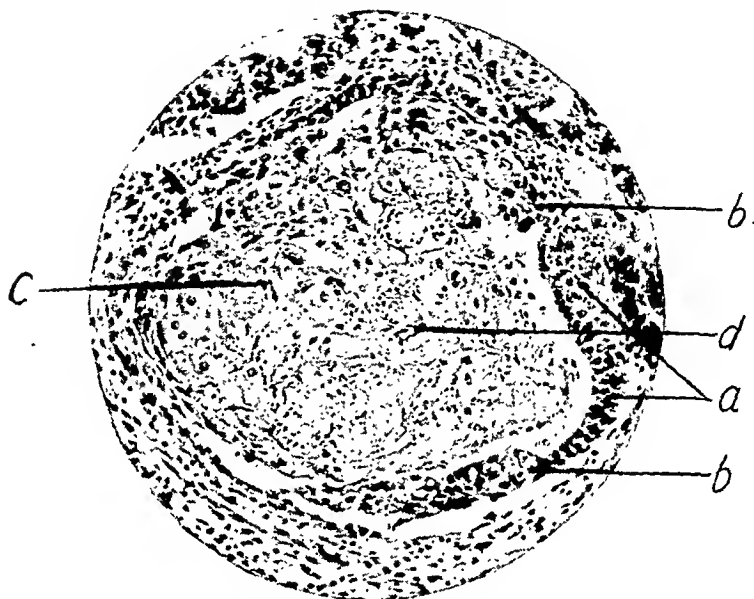


Abbildung 11.

Differenzierung von Drüsenschlauchepithel. a) Proliferation von Epithelzellen, b) Umwandlungsstelle, c) primitive Stachelzellen, d) nekrotisierte Zellen.

der Mitte eines Drüsenschlauches liegenden, behalten meist ihren Kern, wandeln sich aber in der Weise um, dass das ganze Plasma feinkörnig wird, was durch Gram- und besonders durch Kongorot-Färbung als eine beginnende Keratinisierung festgestellt wird (Abb. 10). In anderen der Zellen finden wir dagegen immer deutlicher hervortretende regressive Veränderungen: Plasma- und Kernvakuolisierung, Kernpyknose, Karyorhexis usw. Die Kerne sind schliesslich nur als dünne Schatten zu erkennen, bis sie gänzlich verschwinden. Manche Zellen werden nekrotisch und verschmelzen zu einer zusammenhängenden amorphen Masse (Abb. 9, 11). Derartige Veränderungen konstatieren wir im allgemeinen am meisten in der Mitte des alterierten Zellgewebes, die z. B. bei der Umwandlung des Epithels eines ganzen Drüsenschlauches zugleich der Mitte des gan-

zen Schlauches entspricht. Die weitgehender umgewandelten Epithelpartien sind meist von ihrer Unterlage, dem Bindegewebe, abgelöst; im Zusammenhang mit den Epithelnekrosen ist eine solche Ablösung die Regel.

Die vorstehend beschriebenen Zelltypen und Differenzierungssta-



Abbildung 12.

Übersichtsbild der Differenzierung von Deckepithel. a) Deckepithel, b) Umwandlungsstelle, c) umgewandeltes Epithel, d) keratohyaline Schicht mit Hornbildung, e) umgewandeltes Drüsenschlauchepithel.

dien treffen wir durchaus nicht in allen Drüsenschläuchen an, sondern das Bild ist äusserst bunt und umfasst alle möglichen Übergangsformen von kaum erkennbaren Epithelveränderungen bis zu Verhornungen oder Nekrosen, die den ganzen Drüsenschlauch umfassen (Abb. 9). Schon im Epithel der Drüsenschläuche treten, bevor es sich an umgewandeltes Epithel anschliesst, öfters bedeutende Veränderungen auf. Die Anordnung der Zellen ist gestört, die basalen Zellen liegen wie gewöhnlich senkrecht zu ihrer Unterlage, aber die sie bedeckenden Zellen haben schon eine schräge oder quere Richtung zu den ersteren eingenommen. In den Zellen, besonders den oberflächlichen, bemerken wir oft gleichzeitige deut-

liche regressive Veränderungen: die Kerne sind schlechter färbbar, oft kleiner, pyknotisch, Vakuolisierung tritt sowohl in den Kernen als im Plasma auf. Oft ist das ganze Epithel bedeutend schmaler und zellenärmer als das benachbarte normal erhaltene Epithel (Abb. 9, 10 und 11). An der Übergangsstelle dieser Epithelgebiete, zu Be-

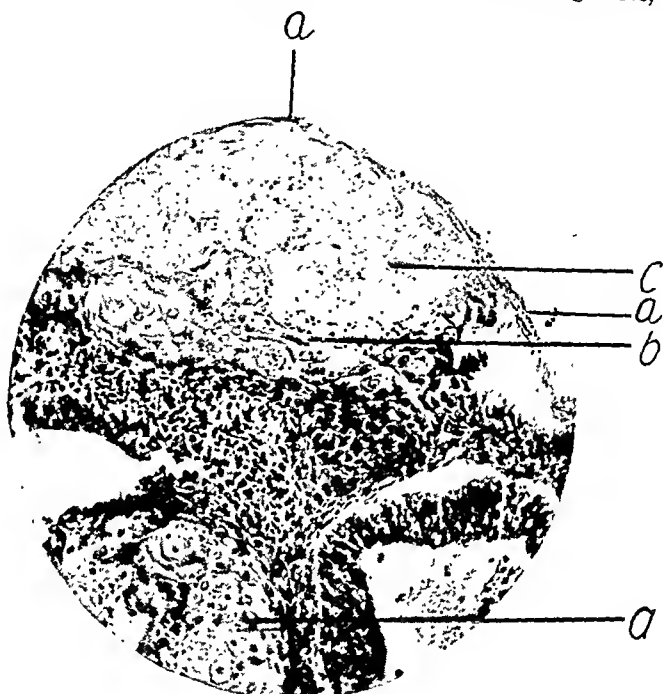


Abbildung 13.

Differenzierung von Deckepithel und Drüschenschlauchepithel.

- a) Deckepithel, b) umgewandeltes Deckepithel mit primitiven Stachelzellen, c) beginnende körnige Keratohyalinisierung, d) umgewandeltes Drüschenschlauchepithel.

ginn der Umwandlung, beobachten wir andererseits oft eine starke Epithelzellenproliferation, wobei die Zellen morphologisch indifferent, von der Form der Basalzelle sind. Namentlich in den tieferen Schichten des Epithelzellengewebes stösst man auf bemerkenswert zahlreiche Zellteilungsfiguren. Dieses Zellgewebe schliesst sich dann, wie oben beschrieben, an weiter differenzierte Epithelschichten an.

Im Bereich des Deckepithels kommt nur an einigen Stellen eine Umwandlung zu Gesicht. Die Umwandlungsstadien gleichen völlig den im Zusammenhang mit dem Drüschenschlauchepithel geschilderten. Die Differenzierung ist jedoch im allgemeinen etwas weiter fortgeschritten (Abb. 12 und 13). So erinnert das Epithelgewebe an

den meisten Stellen an Stachelzellengewebe, obgleich die interzellulären und die Plasmafibrillen recht undeutlich hervortreten und daher nicht von einer regelmässigen Stachelzellenschicht gesprochen werden kann. In den oberflächlichen Zellen ist eine beginnende kleinkörnige Keratohyalinisierung häufig. An einigen Stellen bedeckt schon eine deutliche, wiewohl dünne keratohyaline Schicht das »Stachelzellengewebe«, an gewissen Stellen hinwieder sieht man, dass sich dem Bindegewebe mitten in einem Epithelzug an Hornperlen erinnernde Schichtungen angedrückt haben. Das differenzierte Zellgewebe ist verschieden dick, stellenweise von nur einigen, an anderen Stellen von 10—12 Zellreihen gebildet, und es scheint nicht, wie das Drüsenschlauchepithel, die Neigung zu haben, sich von seiner Unterlage abzulösen oder nekrotisch zu werden. Mitosen treten in dem Basalzellengewebe mässig reichlich auf.

Abrasion II, Probe b). Die Schleimhaut befindet sich in der Sekretionsphase. Die feststellbaren mikroskopischen Befunde gleichen im übrigen genau den oben beschriebenen, nur dass umgewandeltes Epithel bedeutend seltener auftritt und mithin das ziemlich normal erhaltene Epithel häufiger ist. Das umgewandelte Epithel umfasst auch sowohl in den Drüsenschläuchen als im Deckepithel alle Entwicklungsstadien.

Über den Bau, das Vorkommen und die Pathogenese des metaplastischen Plattenepithels in der Schleimhaut des Corpus uteri.

In den oben beschriebenen Fällen haben wir in der Schleimhaut des Corpus uteri vorkommendes Plattenepithel sowohl als Deckepithel wie als von Bindegewebe umgebenes Drüsenschlauchepithel festgestellt. In den drei ersten Fällen vertraten die Epithelinseln ziemlich regelmässiges, allerdings bis zu verschiedenen Differenzierungsstadien fortgeschrittenes Plattenepithel, im vierten Fall dagegen zeigte das Epithel einen besonderen Typus, wies aber doch gewisse für das Plattenepithel kennzeichnende Eigenschaften auf.

Bevor wir uns jedoch der genaueren Betrachtung des Baues und der Pathogenese dieses ortsfremden Epithels zuwenden, müssen wir zuerst unter Beweis stellen, dass es sich in unseren Fällen wirklich um in der Schleimhaut des Corpus uteri auftretendes metaplastisches Plattenepithel gehandelt hat und dass nicht etwa durch embryonale Störungen ent-

standene, von umgebendem Gewebe einwachsende oder durch mechanische Eingriffe, z. B. durch die Operationen, verursachte Plattenepithelformationen und auch nicht Epithel, das nur eine formale Akkomodation des Epithels vertreten würde, vorgelegen haben.

Das dann und wann in der Korpusschleimhaut von Kindern und Neugeborenen vorkommende Plattenepithel ist u. a. von *Meyer* und *Natanson* als eine kongenitale Anomalie betrachtet worden. Ob die in unseren Fällen auftretenden Plattenepithelformationen auch mit Hilfe derartiger von der *Cohnheimschen* Theorie vorausgesetzter embryonaler Missbildungen erklärt werden können, ist eine Frage, die wir zuerst zu entscheiden haben. Wie schon erwähnt, hält *Meyer* diese Möglichkeit wegen der strukturellen Eigenart der sog. Plattenepithelknötchen für nicht ganz ausgeschlossen. Die an unserem Material erhobenen Befunde verleihen jedoch dieser Annahme keine Stütze. In unseren Fällen erscheint das umgewandelte Epithel nämlich erstens bis zu verschiedenen Differenzierungsstadien entwickelt, und zwar so, dass wir in ein und demselben Fall gleichzeitig alle möglichen Übergangsstadien feststellen können, wie es sich z. B. in Fall 4 verhält. Das umgewandelte Epithel ist nicht auf einige Stellen beschränkt, wie man es im allgemeinen bei Kindern konstatiert hat, sondern über die ganze Schleimhaut ausgebreitet, ja stellenweise in dem Masse generalisiert, dass wir kaum Drüenschläuche finden, in denen keine Alteration nachzuweisen wäre (Fall 4). Da wir ausserdem auf Grund der in unserem Fall 4 ausgeführten zweiten Abrasion ersehen, dass die Umwandlung des Epithels wenigstens teilweise in der an dem zyklischen Wechsel teilnehmenden Schleimhaut stattfindet, kann dieses Verhalten ebensowenig wie die von uns gemachten Beobachtungen mit Hilfe von embryonalen Anomalien erklärt werden, sondern die über die Schleimhaut ausgebreitete Epidermisierung muss von Faktoren hergerührt haben, deren Wirkung die ganze Korpusschleimhaut ausgesetzt gewesen ist.

Die Plattenepithelformationen lassen sich auch nicht auf

cin Einwandern oder Einwachsen aus fremder Umgebung, also vor allem aus dem Zervikalkanal oder der Portio zurückführen, wie sie auch nicht auf traumatischem Weg entstanden sein können, denn dann müsste im Wachstum des Plattenepithels eine Ausbreitung per continuitatem und nicht in Differenzierungsstadien verschiedenen Grades in den über die ganze Schleimhaut verzweigten Drüenschläuchen zu konstatieren sein, wie es sich besonders in Fall 4 verhält. In unseren Fällen sind nach der Anamnese auch keine operativen Eingriffe in der Uterushöhle vor der ersten Abrasion ausgeführt worden, wo in allen Fällen schon eine deutliche Alteration der Schleimhaut festgestellt werden konnte.

Und schliesslich können die von uns in der Korpusschleimhaut angetroffenen Plattenepithelinseln auch nicht durch eine nur in der äusseren Form der Zellen im Sinne einer formalen Akkomodation erfolgte Umwandlung verursacht sein, denn das Epithel ist so hoch differenziert, dass es nach der allgemeinherrschenden Auffassung der Pathologen eine Umwandlung des ganzen Zellcharakters voraussetzt.

Wenn wir ausser dem Obigen die in unseren Fällen konstatierten Umwandlungsstadien verschiedenen Grades sowie den nahen, ja stellenweise unmittelbaren Zusammenhang weniger differenzierter Herde mit höher differenzierten Partien in Betracht ziehen, so erklären sich die in unseren Fällen festgestellten Epithelveränderungen nur durch die einzig übrigbleibende Möglichkeit, durch eine wirkliche Metaplasie. Auf die nähere Behandlung dieses Prozesses werden wir später zurückkommen.

Im Bau der metaplastischen Plattenepithelgebiete treten uns bedeutende Unterschiede entgegen. Am weitesten ist die Differenzierung in Fall 2 fortgeschritten. Alle für das Hautepithel charakteristischen Zellschichten waren darin deutlich vertreten. Das Epithel grenzt durch Vermittlung eines oft

scharf hervortretenden, an eine Membrana propria erinnernden Gewebes an das Stroma. Bindegewebspapillen findet man an gewissen Stellen völlig regelmässig entwickelt. Eine Basalzellenreihe kommt mehrerenorts normal zu Gesicht. Die Stachelzellenschicht ist gut entwickelt und umfasst 4—10 Zellreihen. Die Keratohyalinschicht ist von 2—3 Zellreihen gebildet und von einer an einigen Stellen deutlich erkennbaren Hornschicht bedeckt. Die Interzellularfibrillen treten deutlich hervor, und besonders in den basaler liegenden Zellreihen bemerkt man in ihnen oft Bizzozerosche Knoten. Auch der Verlauf der Plasmafibrillen ist bereits der dem Plattenepithel eigentümliche; in den Basalzellen sind die Fibrillen in der Längsrichtung der Zelle, senkrecht zur Oberfläche angeordnet, in der Stachelzellenschicht dagegen mehr tangential zur Oberfläche.

In den Plattenepithelinseln der Fälle 1 und 3 sowie in den meisten des Falles 4 hat die Differenzierung bei einem etwas niedrigeren Stadium haltgemacht. Die Bindegewebspapillen fehlen gänzlich in Fall 1, aber in Fall 3 ist schon eine beginnende Papillenformation festzustellen. Das Epithel grenzt dann und wann an ein einer Membrana propria ähnliches dünnes Gewebe. Die Basalzellenschicht erscheint meist regellos, von polymorphen, polygonalen oder langzylindrischen Zellen gebildet. Dieses Zellgewebe kann auch vollständig fehlen, wobei die Stachelzellenschicht unmittelbar an das Bindegewebe grenzt. Die Dicke der Stachelzellenschicht wechselt stark, zwischen 2 und 10 unregelmässigen Zellreihen, ebenso auch die Form der Zellen. Die interzellulären und intrazellulären Fibrillen sind am deutlichsten in den basalen Schichten zu sehen. Die Anordnung der letzteren Fibrillen ist jedoch meist regellos, dem regellosen Bau des Zellgewebes entsprechend. Eine Verhornung ist in Fall 1 noch nicht zu konstatieren, aber in den anderen Fällen tritt sie schon ungleich weit entwickelt hervor.

Die in Fall 4 vorkommenden Epithelalterationen weichen bedeutend von dem typischen reifen Plattenepithel ab. In ihrem Bau und ihren Zelltypen sind jedoch für das Platten-

epithel wesentliche Eigenschaften festzustellen, obgleich das Epithel nur eine Art unausgebildeten und unregelmässigen Epidermisierungstypus vertritt. In diesem Epithel bemerken wir zuerst die dem Plattenepithel eigentümlichen Zellschichten, allerdings nicht endgültig differenziert, sondern unfertig oder an Übergangsformen erinnernd. Die Basalzellschicht ist oft regellos von polymorphen oder polygonalen Zellen gebildet. An einigen Stellen beobachtet man eine Bildung von Interzellularbrücken, aber nicht sicher eine solche von Plasmafibrillen. Die dieses Gewebe bedeckende Schicht ist ein Übergangsgewebe, dessen Zellen teils Basalzellen, teils Stachelzellen ähnlich sind. Die Zellen sind rund, polygonal oder länglich und meist fest aneinandergeschlossen. Die Kerne und das Plasma sind wohl erhalten. Interzellularbrücken sind augenscheinlich vorhanden, obwohl sie nur an wenigen Stellen undeutlich zu unterscheiden sind. Im Bereich des Deckepithels kann man schliesslich in den äussersten Zellreihen eine deutliche Keratohyalinisierung in Form einer in den Zellen stattfindenden keratohyalinen Granulation wie schon in der Bildung von Hornsubstanz und Ansätzen zu Hornperlen konstatieren. Im Epithel der Drüsenschläuche beobachtet man nur eine beginnende Keratohyalinisierung, d. h. eine kleinkörnige Granulation in Zellen, die weiter von den Basalzellen, oft in der Mitte des am stärksten alterierten Zellgewebes liegen, welche z. B. bei der Umwandlung des ganzen Drüsenschlauchepithels der Mitte des Schlauches entspricht. Die weiter umgewandelten Epithelpartien der Drüsenschläuche haben sich meist von ihrer Unterlage, dem Bindegewebe, abgelöst, eine Neigung, die das alterierte Deckepithel im allgemeinen nicht zu besitzen scheint. In den Epithelpartien der Drüsenschläuche, besonders in den stärker umgewandelten, gewahren wir oft auch regressive Veränderungen von Plasma- und Kernvakuolisierungen, Kernpyknosen und Karyorhexis bis zu vollständigen Nekrosen. Die alterierten Epithelpartien zeigen in unserem Fall keineswegs immer das oben beschriebene Bild, sondern es kann die grösste Buntheit herrschen. Stellenweise umfassen die Veränderungen nur einen Teil von

dem Epithel eines Schlauches, an anderen Stellen einen ganzen Schlauch, ja mehrere sich aneinander anschliessende Schläuche. An gewissen Stellen steht die Umwandlung ganz in den Anfängen und ist kaum erkennbar, an anderen dagegen ist sie bis zu beginnender Verhornung oder vollständiger Nekrose des ganzen Schlauchepithels fortgeschritten.

In Anbetracht des Umfangs des untersuchten Materials dürfen wir wohl das Vorkommen von metaplastischem, hochdifferenziertem Plattenepithel in der Schleimhaut des Corpus uteri als eine verhältnismässig seltene Erscheinung betrachten. Nach meinen Untersuchungen sind nur in drei von tausend Fällen mit Sicherheit Plattenepithelinseln aufgetreten, bei denen die typischen Eigenschaften des Hautepithels nachgewiesen werden konnten. In einem vierten Fall zeigte die Epidermisierung einen speziellen Typus, aber da waren immerhin gewisse für das Plattenepithel charakteristische Eigenschaften zu finden. Die von uns konstatierte Frequenz des Plattenepithels entspricht im grossen und ganzen den von Meyer angeführten Ergebnissen über das Vorkommen der sog. Plattenepithelknötchen, bleibt aber bedeutend hinter der von Deelman beobachteten Häufigkeit zurück.

An Übergangsepithel erinnerndes Gewebe oder Plattenepithel einer niedrigeren Differenzierungsstufe ist dagegen in der Schleimhaut des Corpus uteri bedeutend häufiger anzutreffen. Diese Epitheltypen in der Schleimhaut des Corpus uteri werden im Schrifttum öfters nur als verschiedenartige Atypien des Zylinderepithels erwähnt (vgl. Deelman). In unseren Fällen 1 und 3 haben wir das Vorhandensein derartiger auf niederer Differenzierungsstufe stehenden Epithelgebiete nachgewiesen. Stellenweise haben diese Gebiete an Übergangsepithel mit kubischen Basalzellen erinnert, wobei die diese bedeckenden Zellen grösser und von stärker wechselnder Form, meistens polygonal oder langzylindrisch sind. An anderen

Stellen ist die Differenzierung weiter fortgeschritten, die Interzellularräume treten deutlicher hervor, und besonders in der basalen Schicht konnten schon schwache, aber deutliche Interzellulärbrücken festgestellt werden. Eine Keratohyalinisierung oder eine Bildung von Papillen war in diesen Epithelpartien nie zu beobachten. Die Anordnung der Zellen war im allgemeinen regellos, und die Dicke des Zellgewebes variierte von 1—2 Zellreihen bis zu unregelmässigem Gewebe mit mehreren Zellreihen. Diese Epitheltypen vertraten sichtlich gewisse Differenzierungsstufen der Entwicklungsreihe, die von einem kaum erkennbaren Umwandlungsstadium des Epithels bis zu hochdifferenziertem Plattenepithel führt. Das beweist ausser den bei den verschiedenen Zellgewebstypen konstatierten morphologischen Eigenschaften der nalie, in Fall 1 sogar direkte Zusammenhang, der zwischen den Epithelpartien verschiedener Entwicklungsstadien bestand, und die Tatsache, dass diese Epitheltypen ihr genaues Gegenstück unter den in Fall 4 auftretenden oben beschriebenen Umwandlungsstadien des Epithels finden.

Epithelpartien, die an die vorstehend besprochenen niedrigeren Umwandlungsstadien erinnern, finden sich in der Korpuschleimhaut in dem von mir studierten Material verhältnismässig häufig, ja in Dutzenden von Fällen. Im grössten Teil von ihnen hat es sich offenbar nur um durch verschiedenartige Reize hervorgerufene progressive oder regressive Reaktionen der Schleimhaut, d. h. nur um eine formale Akkomodation des Zellgewebes an die jeweils herrschenden Verhältnisse, nicht um eine Umwandlung des ganzen Zellcharakters gehandelt. An derartiges Übergangsepithel oder vielleicht besser abgeplattetes Epithel erinnerndes Gewebe kam u. a. in zwei von mir untersuchten Fällen von Genitaltuberkulose vor. Hier konnten jedoch bei genauerem Studium dieser Epithelpartien und ihrer Zellen keinerlei auf einen metaplastischen Prozess hinweisende Eigenschaften festgestellt werden. Ähnliche Epithelveränderungen zeigten sich ferner recht zahlreich im Zusammenhang mit Endometritiden sowie bei Frauen jenseits der Menopause, ohne dass in der Schleimhaut der

letzteren auch nur eine schwerere Infektion zu konstatieren gewesen wäre. Solche formale Veränderungen der Schleimhaut des Corpus uteri liegen u. a. in den Serienuntersuchungen *Björkenheims* in mehreren Fällen vor. Ebenso gehören sicher die meisten der z. B. von *Deelman* vorgeführten Atypietypen hierher.

Trotzdem man es in den meisten der von mir untersuchten Fälle nur mit der obenerwähnten formalen Umwandlung des Epithels zu tun gehabt hat, ist es als wahrscheinlich zu betrachten, dass die Veränderungen in einigen doch einen bereits beginnenden metaplastischen Umwandlungsprozess vertreten haben, wiewohl dies in Ermangelung weiter fortgeschrittener Differenzierungsstadien nicht deutlich nachgewiesen werden konnte. Erstens ist ja klar, dass solche höher differenzierten Epithelpartien neben solchen niederen Grades in unseren Fällen öfter vorgekommen sind, als unsere Kasuistik zeigt, obgleich sie zufällig nicht unter unsere Proben geraten sind. Auch ist es möglich, dass die ungleich weit differenzierten Epithelpartien in gewissen Fällen trotz allem Korpusschleimhaut dargestellt haben, wiewohl ich die Fälle kassieren musste, weil sie die gestellten Bedingungen nicht erfüllten. Und schliesslich scheint es mir ausgemacht, dass der metaplastische Prozess unter gewissen Verhältnissen fortgeschritten sein oder in seinen früheren Differenzierungsstadien haltgemacht haben kann. Auch solche Fälle sind sicher zahlreich sowohl unter denen meines Materials als z. B. unter den obenerwähnten von *Björkenheim* und *Deelman* beschriebenen Fällen vorhanden gewesen.

Wenden wir in unseren Fällen die Aufmerksamkeit hiernach dem metaplastischen Prozess selbst zu, so ist gleich hervorzuheben, dass die Untersuchung seines Verlaufs und besonders seiner ersten Phasen Schwierigkeiten bereitet hat. In Fall 2 haben wir neben der übrigen Atypie nicht einmal

deutliches Übergangsepithel, sondern schon relativ weit, aber allerdings in verschiedenem Grade differenziertes Plattenepithel festgestellt. In den Fällen 1 und 3 kommen zwar Gebiete weniger fortgeschrittener Differenzierung des Epithels vor, aber direkte Verbindungsstellen zwischen diesen und dem weiter differenzierten Plattenepithel nur spärlich. An gewissen Stellen (Fall 1) finden wir immerhin, dass ein schon teilweise regressiv veränderter Zylinderepithelzug sowie ein an Übergangsepithel erinnerndes Gebiet sich an eine Plattenepithelinsel anschliessen, aber als vereinzelte Erscheinungen berechtigen diese doch nicht ohne weiteres zu Schlussfolgerungen. In Fall 4 haben wir dagegen allerlei Übergangsformen von leichten Veränderungen bis zum Auftreten von verhorntem Plattenepithel. Die verschiedenen Umwandlungsstadien und der Verlauf des Umwandlungsprozesses waren jedoch in diesem Fall eigenartig und der letztere, wie wir später sehen werden, überdies ausserordentlich schnell, weshalb es schwierig war, die einzelnen Stadien festzustellen; wenn wir aber gleichzeitig die sich langsamer entwickelnden Parallelerscheinungen verfolgen, die *Saxén* eingehend untersucht hat, so ermöglicht sich eine Rekonstruktion des Prozesses.

Erstens können wir konstatieren, dass die Metaplasie in den Drüsenschläuchen, aber wahrscheinlich auch im Bereich des Deckepithels, von einem recht begrenzten Gebiet aus beginnt. Das wird dadurch bewiesen, dass unbedeutende Veränderungen im allgemeinen in Schläuchen festzustellen sind, deren Epithel zum grössten Teil wohl erhalten ist, und dass auch in den am schwersten alterierten Schläuchen ausserordentlich oft noch ziemlich normal beibehaltenes, an seiner Unterlage fixiertes Epithel auftritt (Abb. 9, 10 und 11). An den Stellen beginnender Umwandlung sehen wir zuerst eine starke Proliferation der basal gelegenen Epithelzellen, wobei wir eine Umwandlung zu Zellen konstatieren, welche den Basalzellen oder sonst bezüglich ihres Baues indifferenten Zellen ähnlich sind (Abb. 10 und 11). In diesem neugebildeten Gewebe sehen wir die Differenzierung in der Weise fortschrei-

ten, dass das entstehende Epithelgewebe immer deutlicher den Charakter von Plattenepithel mit den für dasselbe eigentümlichen Zelltypen annimmt.

Ob diese Umwandlung des Epithels so vor sich geht, dass die Ursachen, die zuerst im Zylinderepithel und namentlich in dessen peripher hinreichenden Zellen eine Alteration hervorrufen, dann zugleich in den basal liegenden Zellen zu einer Regeneration führen, wie es *Saxén* auch für die Nasenpolypen nachgewiesen hat, ist eine Frage, die wir zunächst zu entscheiden versuchen müssen.

Für die geäußerte Annahme finden wir in unserem Material eine Stütze. Darauf weisen meines Erachtens zuerst die Befunde hin, die wir über die Ausbreitung des metaplastischen Prozesses in seine Umgebung erheben können. Wie schon bei der Beschreibung unserer Fälle 1 und 4 erwähnt wurde, stellen wir in dem an eine umgewandelte Epithelpartie anschliessenden Zylinderepithel bedeutende Veränderungen fest. Die Zellen dieses Epithels, besonders die peripherer liegenden, sind regellos angeordnet, regressiv verändert, ja oft ist das ganze Epithel stark verdünnt, nur von einer kubischen basal gelegenen Zellreihe gebildet (Abb. 3, 10 und 11). Es scheint auf der Hand zu liegen, dass die Faktoren, die zu den oben erwähnten regressiven Veränderungen geführt haben, zugleich auch eine Regeneration in der basalen Zellschicht bewirken, wobei diese basalen Zellen also zu proliferieren und so neues Epithelgewebe zu bilden anfangen. Diese Proliferation findet natürlicherweise hauptsächlich, ja vielleicht ausschliesslich, in den sich unmittelbar an die umgewandelte Partie anschliessenden Zellen statt, und so setzt sich also das Wachstum des alterierten Gewebes in die Umgebung fort. Da die Aktivität des indifferenten basal gelegenen Zellgewebes zu einer Ausbreitung der so umgewandelten Epithelpartie und auf diesem Wege zugleich zur Bildung eines Zellgewebes von neuem Typus führt, ist die Regeneration, wie *Saxén* auch schon bei den Nasentypen dargelegt hat, als anisogen oder mit anderen Worten als ein Prozess aufzufassen, bei dem die Aktivität der indifferenten

Zellen zur Entstehung von neugebildetem Epithel führt, das seinem ganzen Charakter nach von dem für die Schleimhaut kennzeichnenden Zylinderepithel abweicht. Soweit also die Ausbreitung der alterierten Epithelinseln in ihre Umgebung auf die oben beschriebene Weise vor sich geht, wofür unsere Befunde und *Saxéns* Feststellungen über die von ihm studierten Parallelerscheinungen in jeder Hinsicht sprechen, ist es klar, dass auch die Entstehungsphasen des ganzen metaplastischen Prozesses an den primären Wachstums- oder Umwandlungsstellen die gleichen sind. Dies findet eine weitere Stütze in der Beobachtung, dass wir auch in den beginnenden Umwandlungspartien nie mehr wohlerhaltenes normales Zylinderepithel finden, sondern nur an die basal gelegenen Zellen des Zylinderepithels erinnernde, meist kubische, indifferente Zellen, die z. B. in Abbildung 10 in ringförmiger Kette ein alteriertes Epithelgebiet umgeben und die also das basale Zellgewebe neugebildeten Epithels darstellen. In den weiter fortgeschrittenen Umwandlungspartien sehen wir dagegen oft keine derartigen basal liegenden indifferenten Zellen mehr, sondern schon den basalen Zellen des Plattenepithels ähnliche Zellen, die gerade durch die Aktivität dieser indifferenten Zellen, durch deren Teilung und Umwandlung entstanden sein müssen.

Auf Grund des Obigen erklärt sich, wie mir scheint, die in unseren Fällen konstatierte Umwandlung des Epithels als eine biologische Erscheinung, bei der sowohl regressive als regenerative Prozesse eine Rolle spielen. Die Ursachen oder Reize, die in dem Zylinderepithel, besonders in dessen zur Oberfläche hin reichenden Zellen, eine Alteration, ja einen vollständigen Untergang der Zellen hervorgerufen haben, zwingen zugleich die basal gelegenen und meist wohlerhaltenen Zellen für die Bildung neuen Epithels zu einer regenerativen Aktion. Dieser Regenerationsprozess ist anisogener Art, wobei die Teilung und Proliferation dieser indifferenten Zellen zur Bildung neuen Gewebes führt, dessen Zellen schon mehr oder weniger den Charakter der Hautepithelzellen tragen.

Gehen wir hiernach zu einer eingehenderen Betrachtung des Verlaufs des metaplastischen Prozesses in unseren Fällen über, so können wir gleich konstatieren, dass die Struktur der alterierten Epithelgebiete in unseren Fällen 1, 2 und 3 in den Entwicklungsstadien verschiedenen Grades durchaus die gleiche ist. In diesen Fällen können wir zwar nicht, wie in Fall 4, alle möglichen Entwicklungsstadien feststellen, aber immerhin gewisse Übergangsbilder, die von normalem Zylinderepithel zu Plattenepithel führen, in dem schon alle für diese Epithelart kennzeichnenden strukturellen Eigenschaften nachzuweisen sind. In unserem Fall 2 lässt sich das primärste Entwicklungsstadium nicht konstatieren, wohl aber eine Art Regellosigkeit im Zylinderepithel sowie Differenzierungsstadien verschiedenen Grades, bei denen die Epidermisierung jedoch schon verhältnismässig weit fortgeschritten ist. In unseren Fällen 1 und 3 dagegen bemerken wir ein an Übergangsepithel erinnerndes Entwicklungsstadium, bei dem der metaplastische Prozess schon so weit fortgeschritten ist, dass wir an gewissen Stellen im basalen Zellgewebe schwache, aber doch deutlich erkennbare Interzellularfibrillen auftreten sehen. In Fall 1 erblicken wir ausserdem mehrere Übergangspartien, in denen sowohl ein derartiges an Übergangsepithel erinnerndes Epithel als ein noch die Form des Zylinderepithels bewahrendes regressiv verändertes Epithelgebiet sich an eine hochdifferenzierte Plattenepithelinsel anschliessen und gerade an dieser Stelle in den basalen Zellen des sich umwandelnden Zellgewebes eine starke Proliferation zu beobachten ist (Abb. 3). In diesem proliferierenden Zellgewebe, in dem basalen Zellgewebe des an Übergangsgewebe erinnernden Gewebes sowie des weiter differenzierten Epithels finden wir zahlreiche Mitosen, die einerseits von der Rolle und Funktion der basalen Zellen in diesem metaplastischen Prozess und andererseits von der schnellen Entwicklung des Prozesses zeugen. Während der metaplastische Prozess weiter, wenn auch noch nicht bis zu seinem Endstadium, fortschreitet, weist die Struktur des Zellgewebes noch eine bedeutende Regellosigkeit auf, die sich darin äussert, dass die Form der Zellen, der basalen sowohl

als der Stachelzellen, beträchtlich von der kubischen oder polygonalen bis zur langzylindrischen variiert, dass der Verlauf der Plasmafibrillen regellos ist und dass die Anordnung der Zellen oft völlig verworren erscheint. In den am höchsten differenzierten Gebieten (in Fall 2, Abb. 5 und 6) finden wir die Epithelstruktur schon durchaus regelmässig und das Epithel mit allen für das Hautdeckepithel wesentlichen morphologischen Eigenschaften ausgestattet.

Obwohl wir nicht in jedem einzelnen unserer Fälle mehrere verschiedene Entwicklungsstadien untersuchen konnten, ergänzen sie einander doch und bilden miteinander eine zusammenhängende Entwicklungsreihe, in der sich der Verlauf des metaplastischen Umwandlungsprozesses als genau dem oben dargestellten konform erwiesen hat und der also für die Aufhellung der Genese der Metaplasie von grosser Bedeutung ist.

Das am weitesten differenzierte metaplastische Plattenepithel, das in allen diesen Fällen vom gleichen Typus ist und das, der Struktur nach zu urteilen, das Ergebnis einer langen Entwicklung darstellt, repräsentiert in den zwei ersten Fällen die Umwandlung des Epithels in der Uterusschleimhaut, die nicht mehr zyklischen Wechseln unterworfen gewesen ist, während die zyklische Funktion in Fall 3 noch besteht, obwohl sie schon längere Zeit, wie auch die Metropathie der Patientin zeigt, erheblich gestört gewesen ist. Es ist denn auch offenbar, dass dieser letzterwähnte Störungszustand der Schleimhaut an seinem Teil die Verhältnisse geschaffen hat, unter denen die Entwicklung des metaplastischen Prozesses auf dieselbe Weise und bis zu demselben Grade fortschreiten konnte wie bei Frauen nach der Menopause und also, nach der Struktur zu schliessen, eine regelmässigere, länger dauernde und beständigere Epidermisierung erreicht wurde, was im allgemeinen in der regelmässig zyklisch funktionierenden Schleimhaut kaum als möglich betrachtet werden könnte.

Die in unserem Fall 4 festgestellten Umwandlungsstadien, die in allen möglichen Übergangsformen von normalem Zylind-

derepithel bis zu schon eine keratohyaline Schicht umfassendem neuen Epithel auftreten, sind dazu angetan gewesen, unsere frühere Aufklärung der Anfangsstadien des metaplastischen Prozesses und mithin teilweise der Genese selbst weiter zu fördern, weshalb wir in diesem Zusammenhang nicht mehr direkt auf diese Frage eingehen. Im Verlauf des metaplastischen Prozesses selbst tritt dagegen eine so grosse Eigenart auf, dass er ganz besondere Aufmerksamkeit verdient.

Auf Grund der früheren Behandlung dürfte schon klar geworden sein, dass die Alteration, ja der vollständige Untergang der peripheren Zellen des Zylinderepithels die basal gelegenen Zellen zu einer regenerativen Aktion geführt hat, um neues, den Verhältnissen besser angepasstes Epithel zu schaffen. Dieser regenerative Prozess verläuft augenscheinlich recht schnell (vgl. *Hamperl*). Das zeigen die ausserordentlich zahlreichen und äusserst mannigfaltigen Zellteilungsfiguren im basalen Zellgewebe sowie auch die Tatsache, dass das basale Zellgewebe in den weiter umgewandelten Partien verhältnismässig bald seine typische Form verliert und an manchen Stellen durch ein Gewebe aus Zellen, die gewissermassen an Stachelzellen erinnern, ersetzt wird. In diesem metaplastischen neuen Gewebe ist jedoch besonders auffallend, dass wir wohl im ganzen Gebiet unserer Proben kaum eine einzige in ihrer Zellstruktur regelmässig differenzierte Stachelzelle finden können, sondern nur eine Art abortiv entwickelter Zellen, die jedoch gewisse Eigenschaften der Stachelzelle oder des Stachelzellengewebes besitzen, wie z. B. schon an einigen Stellen feststellbare, aber schwach entwickelte Interzellularbrücken. Die Keratohyalinisierung tritt auch, von gewissen im Bereich des Deckepithels konstatierten deutlichen Schichtungen abgesehen, nur als beginnende kleinkörnige Granulation auf. Die erwähnten Erscheinungen erklären sich wohl am ehesten aus der Schnelligkeit, mit der diese ganze eigenartige, abortive Epidermisierung offenbar stattfindet. Die im Gebiet des Drüsenschlauchepithels auftretenden zahlreichen Nekrosen beruhen augenscheinlich ihrerseits auf der Neigung der umgewandelten Epithelpartie, sich von ihrer Unterlage

abzulösen, eine Neigung, die das Deckepithel im allgemeinen ebensowenig zu besitzen scheint, wie es zum Nekrotischwerden tendiert.

Betrachten wir die Ursachen, die zu diesem metaplastischen Prozess von eigenartigem Typus führen, so müssen wir vor allem die Verhältnisse beachten, unter denen die Umwandlung stattfindet. Hierbei konstatieren wir zuerst, dass die Patientin lange dauernde regelmässige Ausflüsse gehabt hat und dass in Probe a) in der Uterusschleimhaut deutliche metropathische Veränderungen vorhanden gewesen sind, so dass die zyklische Funktion der Schleimhaut erheblich gestört gewesen sein muss. Vor der Entnahme der Probe b) dagegen konnten in den Menstruationen keinerlei Störungen nachgewiesen werden, sondern der regelmässige zyklische Ausfluss trat im Gegenteil zwei Wochen vor der Abrasion auf. Da aber doch auch in dieser Probe die meisten Epithelveränderungen schon bis zu ihrem Endstadium differenziert sind, ist meiner Ansicht nach die Annahme voll berechtigt, dass dieser metaplastische Prozess dem zyklischen Wechsel der Schleimhaut gefolgt ist und dass mithin die weit fortgeschrittene Differenzierung während des Proliferationsstadiums der Schleimhaut, d. h. im Lauf einer verhältnismässig kurzen Zeit stattgefunden haben muss. Bedenken wir, dass in beiden Proben die umgewandelten Epithelpartien in allen Entwicklungsstadien bezüglich der Struktur des Zellgewebes, der morphologischen Eigenschaften der Zellen und des Verlaufs des Prozesses völlig gleichartig sind, so ist es offenbar, dass auch die in Probe a) auftretende Umwandlung in Schleimhaut vor sich geht, die wenigstens teilweise in zyklischer Funktion begriffen ist. Da die Desquamationsphase der Uterusschleimhaut nicht normalerweise vor der Entnahme der Probe a) stattgefunden hat, wie es sich bei den Metropathien verhält, ist die Schleimhaut mit ihren alterierten Epithelpartien auch bei weitem nicht immer allmonatlich abgegangen, sondern das umgewandelte Epithel ist stationärer geblieben, was wiederum seinesteils zu den so häufig in ihr konstatierten Nekrosen geführt hat.

Aus dem Obigen dürfte hervorgegangen sein, dass sich der

metaplastische Umwandlungsprozess in unserem Fall 4 wenigstens teilweise in einer an der zyklischen Funktion teilnehmenden Schleimhaut entwickelt hat. Betrachten wir demgegenüber die in unserem Fall auftretende besondere Umwandlungserscheinung, so erhalten die abortive Struktur des neugebildeten Gewebes, die unausgebildeten morphologischen Eigenschaften der Zellen und die eigenartige und schnelle Entwicklung des ganzen Prozesses eine natürliche Erklärung. Dieser ganze metaplastische Differenzierungsprozess dürfte also nur den speziellen Umwandlungstypus vertreten, der in Schleimhaut mit zyklischer Funktion möglich wird und dem die herrschenden Verhältnisse so einen eigenen speziellen Charakter verleihen.

Der von uns festgestellte schnelle Verlauf dieses zyklischen metaplastischen Prozesses ist geeignet, Einwendungen hervorzurufen. Es kann nämlich in Frage gestellt werden, ob der metaplastische Differenzierungsprozess sich überhaupt unter irgendwelchen Verhältnissen in so kurzer Zeit, in einer oder zwei Wochen, zu seinem Endstadium entwickeln kann. Die Entwicklungsdauer des metaplastischen Prozesses beim Menschen zu bestimmen, ist natürlich ausserordentlich schwer. Darum habe ich auch in den früheren Untersuchungen keine direkte Stütze für die von uns konstatierte Erscheinung gefunden. In Tierversuchen begegnen wir dagegen diesbezüglich deutlichen Hinweisen. So haben z. B. *Wolbach* und *Howe* in den Schleimhäuten der von ihnen studierten Versuchstiere, und zwar auch in der Uterusschleimhaut, bei Fütterung der Tiere mit völlig A-vitaminfreier Kost eine Bildung von metaplastischem Plattenepithel hervorgerufen. Dieses trat nach verschieden langer Zeit auf. Die Anwendung von A-Vitaminen hinwieder führt zum Ersatz des metaplastischen Plattenepithels durch normales, der betreffenden Schleimhaut eigentümliches Epithel. Die ersten Symptome der Regeneration des Epithels sind schon am fünften Tage und die vollständige Regeneration, die jedoch in den Schleimhäuten der verschiedenen Organe einigermassen variiert, etwa zehn Tage nach Beginn der Behandlung zu beobachten. Für die Regene-

ration sind also 10 Tage erforderlich gewesen, obwohl das metaplastische Plattenepithel in mehreren Fällen stark keratinisiert war. Dieselbe Zeit genügt offenbar auch für die metaplastische Umwandlung. Obgleich die Regeneration der Schleimhaut beim Menschen langsamer erfolgt, ist die Proliferationsphase auf Grund des obigen Befundes in unserem Fall entschieden als für die Entwicklung des da auftretenden abortiven metaplastischen Plattenepithels hinreichend zu betrachten.

Bei der Analyse der Ursachen oder Reize, die zur Entstehung von Metaplasien in der Uterusschleimhaut führen können, müssen wir unser Augenmerk auf die krankhaften Erscheinungen richten, bei denen Metaplasien festgestellt worden sind. Von rein traumatischen Narbengeweben abgesehen, wird in der Literatur das Vorkommen von Plattenepithel im Korpus Erwachsener manchmal im Senium, in gewissen Fällen bei Uterussarkomen, aber hauptsächlich nur im Zusammenhang mit verschiedenartigen Infektionen erwähnt.

Der Anteil traumatischer Faktoren ist in unseren Fällen schon durch die früheren Darlegungen eliminiert worden. Ebenso konnte in unseren Fällen auch weder Sarkom noch Lues oder Tuberkulose konstatiert werden. Die Bedeutung der Tuberkulose als ätiologischer Faktor ist u. a. von *Franqué*, *Alterthum*, *Sitzenfrey* und *Björkenheim* hervorgehoben worden. Ebenso wie *Deelman* habe ich auch in zwei Fällen meines Materials in der Mukosa des Korpus in der Nachbarschaft eines tuberkulösen Prozesses oder an einen solchen anschliessend plattenepithelähnliche Partien festgestellt. Bei näherer Untersuchung haben sich diese jedoch nur als reaktive Akkomodationsprodukte und nicht als Metaplasien herausgestellt.

In allen Fällen meines Materials war dagegen eine inflammatorische Infiltration in der Mukosa zu sehen. In den Fällen 1, 2 und 4 war sie ausserordentlich stark, in den beiden ersten fast ausschliesslich von Leukozyten verursacht, und auch in dem letzten standen die Leukozyten in den schwerer alterier-

ten Partien im Vordergrund. In Fall 3 waren die Infiltrationszellen hauptsächlich von Plasmazellen und Lymphozyten gebildet, aber unter diesen fanden sich auch reichlich Leukozyten. In den beiden ersten Fällen ist ein sehr reichliches Rundzelleninfiltrat überall in die Gewebe, bis in die Höhlen zwischen den metaplastischen Epithelzellen eingedrungen. Wenn man ausserdem in Betracht zieht, dass in Fall 4 Stellen vorhanden sind, wo die Zellinfiltration verhältnismässig gering ist, dass aber überall, wo die Epithelveränderungen schwerer sind, auch Exsudatzellen sowohl in der Umgebung als im Bereich des umgewandelten Epithels überaus reichlich auftreten und dass die Leukozyten in diesem Gebiet in grossen Gruppen in einen Drüsenschlauch und in dessen Epithel, in das metaplastische Zellgewebe und die Nekrosen eingedrungen sind, so scheint es wahrscheinlich, dass die Infektion, die Bakterientoxine, einen, in unseren Fällen sogar vielleicht den wichtigsten Reiz zur Entstehung der Metaplasie darstellen. Vielleicht sind gerade diese Bakterientoxine und die leukozytäre Infiltration, indem sie Alteration, ja vollständigen Untergang des Epithels oder seiner oberflächlicheren Zellschichten herbeiführen, die Ursache dazu, dass das erhaltene oder im Bereich der Erosion neugebildete Basalzellengewebe bei seiner Regeneration gezwungen gewesen ist, sich den veränderten Verhältnissen in der Weise anzupassen, dass vielleicht besser zu den herrschenden Verhältnissen stimmendes Gewebe entsteht, das in diesem Fall Plattenepithel ist.

Es versteht sich von selbst, dass die Bakterientoxine und die Exsudatzelleninfiltrationen nicht allein zur Entstehung von Metaplasien führen, denn wenn das der Fall wäre, wären die metaplastischen Prozesse mehrmals häufiger, sondern ausser und neben der Infektion sind offenbar eine Anzahl Faktoren wirksam, die wir nicht genauer kennen, von denen wir aber wohl doch einige anführen können.

Als ein solcher spezifischer Faktor oder Zustand ist erstens das Senium oder der Zustand der Korpusschleimhaut zu betrachten, bei dem die zyklische Funktion schon aufgehört hat. Bei den betreffenden Individuen ist denn auch das Vor-

kommen von Plattenepithel im Korpus sowohl im Zusammenhang mit einer Infektion als bisweilen auch ohne eine solche am häufigsten gewesen. Eine derartige ihrer zyklischen Funktion entkleidete, in gewisser Weise alterierte Schleimhaut ist offenbar mit der in anderen Organen und Höhlen auftretenden beständigen Schleimhaut zu vergleichen, in der die Metaplasien auch bedeutend häufiger als in dem normal funktionierenden Uterus sind. Von den Fällen unseres Materials gehören die zwei ersten zu dieser Gruppe.

Die hormonalen metropathischen Veränderungen der Korpus Schleimhaut, die wir u. a. in unseren beiden letzten Fällen konstatiert haben, spielen augenscheinlich bei der Entstehung der Metaplasien eine Rolle. Bezeichnet dies doch einen Zustand, bei dem die zyklische Funktion der Schleimhaut schwer gestört ist, bei dem, von der Hypertrophie der Schleimhaut abgesehen, das Epithel sich lange Zeit erhalten und den verschiedenartigsten regressiven und regenerativen Veränderungen ausgesetzt werden kann, was alles dazu angetan ist, die Entstehung von Metaplasien zu erleichtern. Diese Auffassung wird auch dadurch bestätigt, dass die Schleimhaut in Verbindung mit den sog. Plattenepithelknötchen ziemlich regelmässig verdickt, hypertrophisch gefunden worden ist. Diese Verdickung der Schleimhaut hat offenbar in den meisten Fällen gerade eine metropathische Hypertrophie dargestellt, d. h. hauptsächlich eine von vermehrter und fortgesetzter Follikulin- und mangelnder oder sparsamer Lutinsekretion herührende Störung in der Entwicklung der Schleimhaut, obgleich die Schleimhautveränderungen lange nicht in allen veröffentlichten Fällen genauer beschrieben worden sind. Ferner wird die von uns angeführte Auffassung u. a. durch die Untersuchungen von *Migliacca*, *Gumbrecht* und *McEuen* an Versuchstieren unterstützt. Durch längere Anwendung reichlicher Mengen Follikelhormon haben sie nämlich bei ihren Versuchstieren eine an Metropathie erinnernde Verdickung der Schleimhaut und zugleich eine Bildung von metaplastischem Plattenepithel in der Korpus Schleimhaut hervorgeufen.

Das Obige berechtigt wohl zu der Schlussfolgerung, dass die metropathische Hypertrophie der Korpusschleimhaut, die ein längeres Erhaltenbleiben des Epithels und zugleich auch dessen Alteration und Regeneration bedeutet, eine und zwar vielleicht eine ganz entscheidende Grundbedingung für die während der zyklischen Funktion des Uterus im Epithel stattfindende metaplastische Umwandlung darstellt.

Wie schon oben in gewisser Weise gesagt wurde, sind die Avitaminosen und vor allem der Mangel des Vitamins A für die Entstehung der Metaplasie von Bedeutung. Haben doch Wohlbach und Howe gezeigt, dass der Mangel des Vitamins A bei Ratten zur Alteration und Atrophie der Schleimhäute und schliesslich zur Bildung von metaplastischem Plattenepithel führt. Diese Befunde sind zwar nur an Versuchstieren erhoben, da aber die Reaktionsweise des Organismus mit der Entwicklung im allgemeinen die gleiche bleibt, darf man bei den genannten Mängeln ein Auftreten entsprechender Erscheinungen auch in den Schleimhäuten des Menschen als sicher ansehen.

In den oben beschriebenen Fällen haben also Hormon- und Vitaminstörungen zu Hypertrophie oder Atrophie der Schleimhäute und zugleich durch im Epithel auftretende verschiedenartige regressive und regenerative Prozesse zu metaplastischen Erscheinungen geführt. Obwohl die Störungen im Vitamin- und Hormonhaushalt deutlich zur Bildung von Metaplasien beitragen, dürfte das doch nur als etwas Mittelbares, Bodenbereitendes, d. h. in der Weise zu verstehen sein, dass diese Störungen in den Schleimhäuten einen Zustand schaffen, bei dem das Epithel länger erhalten bleibt und bei dem das alte Epithel oder die zur Oberfläche hin reichenden Zellen des Epithels alteriert werden und die basale Zellschicht bei seiner Regeneration gezwungen ist, Gewebe, Plattenepithel, zu bilden, das besser zu den neuen, jeweils herrschenden Verhältnissen passt. *Soweit es sich so verhält, ist es augenscheinlich, dass auch alle anderen Prozesse, die zu länger dauernden Hypertrophien oder Atrophien mit den obenerwähnten Epithelveränderungen führen, dazu angetan sind, die Entstehung*

von Metaplasien zu erleichtern. Von derartigen Fällen seien als Beispiele angeführt z. B. die verdickte, ja polypöse Schleimhaut, die die Unebenheiten oder Buchten der von submukösen Myomen gebildeten Korpushöhlen ausfüllt, die sog. Metropathia ex vacui, und andererseits die durch Myome oder andere Ursachen hervorgerufenen Druckatrophien oder die von Allgemeinkrankheiten herrührenden sonstigen zu Atrophie führenden Prozesse in der Korpusschleimhaut. Ferner kann man sich als einen für Metaplasie disponierenden Faktor, wie bei den Nasenpolypen, einen mechanischen Reiz denken, dem die Schleimhaut z. B. bei gestielten submukösen Myomen ausgesetzt werden kann.

Von den oben geltend gemachten Gesichtspunkten abgesehen, gibt es im Organismus und seiner Funktion natürlich zahlreiche Faktoren und Störungen, die bei der Entstehung der metaplastischen Prozesse eine Rolle spielen können, deren Art wir aber noch nicht kennen.

Schliesslich ist es am Platze, kurz auf *die Frage von der genetischen Beziehung des metaplastischen Plattenepithels zu dem Karzinom* einzugehen. Obwohl Meyer und Deelman in der Korpusschleimhaut nie einen Zusammenhang mit im Korpus vorkommenden Plattenepithelkarzinomen, Kankroiden, feststellen konnten, ist doch theoretisch die Möglichkeit dazu vorhanden. Diese Möglichkeit unterstreichen u. a. Hofmeier, Piering, Pfannenstiel und Gebhard, obgleich sie nicht imstande gewesen sind, sie bindend zu beweisen. Eine solche karzinomatöse Umwandlung hat man denn auch in den mit Zylinderepithel bedeckten Schleimhäuten anderer Organe und in Papillomen konstatieren können; z. B. im Darmkanal (Hauser), in der Harnblase (Stenius), in der Nasenhöhle (Saxén) und in experimentellen Papillomen bei Tieren (Fibiger, Deelman u. a.). *Es ist offenbar, dass die Korpusschleimhaut in dieser Hinsicht keine Ausnahme macht, sondern dass die karzinomatöse Umwandlung des metaplastischen Platten-*

epithels einmal bei späteren Untersuchungen nachgewiesen wird.

ZUSAMMENFASSUNG.

Die vorliegende Untersuchung über die Schleimhaut des Corpus uteri fusst auf 1000 Fällen. In drei Vierteln war Curettenmaterial Gegenstand der Untersuchungen, in den anderen Fällen bei den Operationen entnommene Gewebsstückchen. Besondere Aufmerksamkeit wurde der Differenzierung der Korpusschleimhaut von der Schleimhaut der Umgebung unter anderem mit Hilfe der Muzinfärbung zugewandt. Übergangsepithel oder an eine niedrigere Differenzierungsstufe des Plattenepithels erinnerndes Epithel fand sich in der Korpusschleimhaut verhältnismässig oft, ja in Dutzenden von Fällen. In den meisten handelte es sich jedoch nur um eine formale Akkomodation der Epithelzellen, aber in einem Teil schon sicher um eine Differenzierung des Epithels im Frühstadium. Nur in vier Fällen wurde das Vorkommen von metaplastischem Plattenepithel in der Schleimhaut sowohl als Deck- wie als Drüsenschlauchepithel festgestellt. In drei Fällen vertraten die metaplastischen Plattenepithelinseln ziemlich regelrechtes, allerdings bis zu einem verschiedenen Differenzierungsstadium fortgeschrittenes Plattenepithel, worin in den weitest entwickelten Inseln die für das Plattenepithel kennzeichnenden Zellschichten konstatiert wurden, d. h. eine typische Basalzellschicht, von Stachelzellen gebildete Schichten, eine keratohyaline Zellschicht mit Horngebildebildung sowie in einem Fall auch schon eine Bildung von Bindegewebspapillen. In dem vierten Fall liess das umgewandelte Epithel einen Sondertypus erkennen. Auch da wurde festgestellt, dass das neugebildete Epithel gewisse für das Plattenepithel wesentliche Eigenschaften besass, nämlich neben einem von primitiven Basalzellen und schon mit schwach entwickelten Interzellularbrücken versehene Stachelzellen stellenweise bereits ziemlich typische keratohyaline Schichten und Hornperlen.

Die verschiedene Differenzierungsstufen vertretenden Epithelinseln, die an gewissen Stellen unmittelbar aneinanders stiessen, sowie in Fall 4 das gleichzeitige Vorkommen der verschiedenartigsten Übergangsformen und Entwicklungsstadien in ein und derselben Gewebprobe gestatteten, die Entwicklung des metaplastischen Differenzierungsprozesses von seinen Anfangsphasen bis zur Bildung einer Hornschicht zu studieren. Die Umwandlung begann in verhältnismässig begrenzten Gebieten. An der Umwandlungsstelle wurde zunächst eine Alteration der zur Oberfläche hin reichenden Epithelzellen bis zu ihrer Zerstörung gefunden, wobei die basalen Zellen ihre frühere Grösse und Form beibehielten. Dieselbe Ursache, durch welche die Zerstörung der oberflächlichen Zellen bewirkt worden ist, hat dann zur Tätigkeit der indifferenten basalen Zellen, d. h. zur Bildung von neuem, den Verhältnissen besser angepasstem Epithel geführt, das sich in unseren Fällen als Plattenepithel mit den für es typischen Eigenschaften erwies. Der Verlauf des Differenzierungsprozesses war in den drei erstgenannten Fällen langsamer und regelmässiger, so dass die verschiedenen Zellschichten die für das Plattenepithel charakteristischen morphologischen Eigenschaften erlangt haben. In dem vierten Fall andererseits, in dem die Schleimhaut wenigstens noch teilweise ihre zyklische Funktion beibehalten hat, ist die Differenzierung, auch nach den zahlreichen Kernteilungsfiguren zu urteilen, schnell vor sich gegangen. So haben sich keine typische Basal- und keine Stachelzellenschicht gebildet, sondern die Zellen haben sich nur zu an diese erinnernden abortiven Zellen entwickelt, wie die Keratisierung neben den Schichtungen auch oft in Form einer primitiven Körnung aufgetreten ist. Der schnelle Verlauf des Prozesses hat oft auch zu Nekrosen des neugebildeten Epithels geführt, die zahlreich Hand in Hand mit Entwicklungsstadien verschiedener Phasen angetroffen wurden. Diese schnelle und eigentümliche Differenzierung vertrat den metaplastischen Umwandlungsprozess, der in der noch an der zyklischen Funktion teilnehmenden Schleimhaut möglich geworden ist.

Über die zur Bildung von metaplastischem Plattenepithel führenden Ursachen konnten an unserem Material eine Anzahl Beobachtungen gemacht werden. Exsudatzelleninfiltration in der Mukosa wurde in allen Fällen konstatiert. Diese Infiltrationen und Bakteritoxine lösen jedoch nicht allein die Bildung von metaplastischem Plattenepithel aus, sondern neben ihnen konnten gewisse andere Faktoren nachgewiesen werden, die sicher bei der Entstehung dieses Prozesses eine Rolle spielen. Als solcher Spezialfaktor ergab sich erstens das Senium oder der Zustand der Mukosa, in dem die zyklische Funktion schon aufgehört hat. Zu dieser Gruppe gehörten die zwei ersten Fälle. In den zwei letzteren Fällen hinwieder haben die auf hormonalen Funktionsstörungen beruhenden metropathischen Schleimhautaffektionen des Corpus uteri eine offenbar entscheidende weitere Ursache zur Entstehung der Metaplasie gebildet. Von diesen weiteren Faktoren seien ferner die in der Behandlung des Materials angeführten hormonalen Störungen, der A-Vitaminmangel, mechanische Reize usw. erwähnt.

In dieser Arbeit konnte ebensowenig wie in früheren Untersuchungen mit Sicherheit das genetische Verhältnis des metaplastischen Plattenepithels in der Schleimhaut des Corpus uteri zu dem Karzinom nachgewiesen werden. Es ist augenscheinlich, dass die Schleimhaut des Corpus uteri in dieser Beziehung keine Ausnahme von den anderen Schleimhäuten macht, sondern dass die karzinomatöse Umwandlung auch dieses metaplastischen Plattenepithels einmal bei späteren Untersuchungen festgestellt werden wird.

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THE IN VITRO ACTION OF TRICHINA LARVAE IN IMMUNE SERUM — A NEW PRECIPITIN TEST IN TRICHINOSIS.

By *Hans Roth*.*)

(Received for publication December 7th, 1940).

In 1938 *Sarles* demonstrated that precipitates developed on the cuticula, at the mouth, at the excretory pore and in the intestine of larvae of *Nippostrongylus muris*, an intestinal nematode of the rat, when they were incubated in rat immune serum (i. e. serum of rats which, after repeated infections with this worm, had acquired immunity to reinfection). Recently, similar observations were made for the larvae of *Ancylostoma caninum*, the hookworm of the dog, by *Otto* (1940) and for those of *Strongyloides ratti*, a parasite of the rat, by *Lawler* (1940). *Sarles* and *Otto*, at any rate, saw also some inhibitory effect from these precipitates formed in immune serum on the ability of the larvae to develop, and all three authors assume a close correlation between this in vitro action of the immune serum and its protective properties in vivo, substantiated by passive transfer of some degree of immunity to other animals.

Already in 1917 *Schwartz* tested the in vitro effect of specific immune serum on the larvae of *Trichinella spiralis*, but without results. The present author has examined this question again, employing the following procedure:

*) The author wishes to express his gratitude to the Rask-Ørsted Fund, Copenhagen, for a fellowship which made possible this investigation.

The infective trichina larvae were obtained from trichinous muscles of rabbits or guinea pigs (at least 8 weeks after infection) by artificial digestion, the meat being added, in the ratio of 1 to 20, to an aqueous solution of 0.5 per cent HCl and 1.0 per cent pepsin. This mixture was incubated for 12 hours at 37° C and then stirred constantly for another 4 hours, also at 37° C, whereafter it was poured through a 30-per inch wire screen into a large funnel, to the neck of which was attached a short rubber tube closed with a clamp. After standing for about 2 hours most of the larvae, freed from their cysts, but still well coiled up, had collected at the bottom of the funnel and could be drawn off by opening the clamp. They were then washed several times first with tap water and later with sterile saline so as to clean them as far as possible from adherent bacteria.

For each sample, about 100 larvae were placed in the depression of a sterile hollow-ground slide (3.5 mm thick) and covered with nearly 0.5 cc of sterile unheated serum. Then, a sterile coverslip (24 × 32 mm) was laid over the depression, whereby care was taken to prevent air bubbles passing in. Finally, the coverslips were bordered with sterile vaseline. The preparations were incubated at 37° C and examined at intervals under the microscope.

Altogether, 12 sera of heavily trichinized guinea pigs, 7 of heavily infected rabbits and 26 human sera (from abroad) from patients suffering from clinical trichinosis were tested, many of them several times.

After from 3 to 6 hours, more or less distinct, bubble-like precipitates were seen at the anterior ends of many of the larvae incubated in immune serum. During the following hours, these precipitates increased in size and multiplied, so that sometimes a whole appendage of conglomerating precipitates could be seen adhering to the anterior end (fig. 1). The larvae were very active, waving their fore-ends in attempts to get rid of the adherent masses, and were almost always successful. After from 16 to 24 hours, the serum thus contained larger or smaller quantities of such free, cast-off precipitates. These could be sharply formed as more or less regular bubbles of different size (fig. 2), which was particularly the case in very potent sera, especially those of rabbits or of man, while in other sera, particularly in those of lesser potency (as e. g. those of guinea pigs), rather amorphous, finely granular pre-

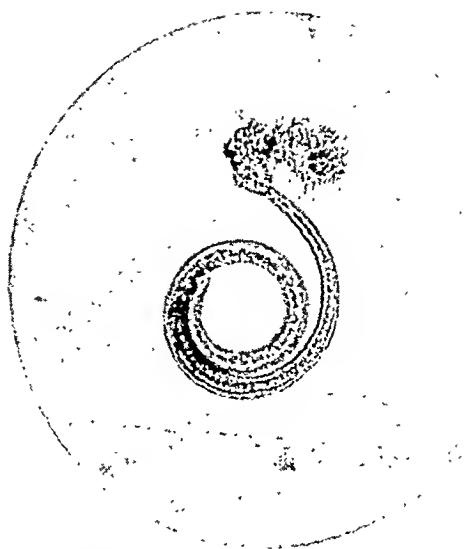


Fig. 1. Photomicrograph of a trichina larva which shows, adhering to its fore-end, an appendage of conglomerating precipitates. After 24 hours' incubation at 37° C in the serum of a rabbit, heavily trichinized 10 weeks before. ($\times 100$).



Fig. 2. Photomicrograph of trichina larvae surrounded by free, mostly bubble-shaped precipitates. After 24 hours' incubation at 37° C in the serum of a patient who has been suffering from trichinosis for more than 2 months. ($\times 100$).

precipitates were predominant. Even in those cases where bacterial contamination interfered, it was often possible to distinguish between the bacterial growth and the precipitates.

To sum up, of the 12 sera of infected guinea pigs, which were examined between the 56th and the 264th day after infection, 10 yielded positive results, while the reaction of the other 2 was dubious. All the 7 trichinized rabbits were positive when they were tested between the 14th and the 454th day after infection, while the examination, already on the 7th day, of 3 of them resulted in a weak, positive reaction in the first, in a dubious reaction in the second, and in a negative in the third. Finally, all the 26 human sera which were tried about two and a half months after the epidemic had broken out, yielded definite positive results, though the potency of the individual sera was, obviously, somewhat varying. 6 control sera of uninfected guinea pigs, 9 control sera from rabbits, and 6 human control sera did not show reaction.

In a special experiment, 0.1 per cent formaldehyde was added to the larvae to inhibit bacterial growth. Although the vitality of the worms was not visibly influenced, no precipitates occurred in the immune sera, and the method had therefore to be abandoned. In another experiment, it was tried if the metabolic products of the larvae were alone able to evoke the specific effect. A great number of trichina larvae were incubated for 20 hours in sterile saline at 37° C. Then the suspension was filtered through a No. 3 Seitz filter, and some drops of the filtrate were mixed on the hollow-ground slides with some very potent immune sera. After 24 hours' incubation at 37° C no reaction was seen. This must lead to the conclusion that it is the immediate interaction between the living larva itself (which apparently in serum secerns an oral secretion of antigenic power) and the immune serum, which gives rise to the precipitation of the antibodies present.

In contrast to the observations made with *Nippostrongylus muris* and *Ancylostoma caninum*, no deleterious effect of the precipitates on the larvae was seen. Mostly the larvae showed, both in immune and in normal serum, intense shrinkage of the

posterior end after 24 hours' incubation. This was obviously an unspecific reaction occurring in serum since it was not seen in larvae that had been incubated in saline. On the other hand, when trichina larvae which had been immersed for 2 hours in immune serum at room temperature, were fed to guinea pigs, the resulting infestation of the muscles was just as great as when the same number of larvae immersed in normal serum or in saline was ingested. In this connection, it is also worth stressing that opinions differ greatly as to the protective value of immune serum in trichinosis. The present author (in experiments to be published later) has not as yet seen any noticeable effect of injections of immune serum on the course of the infection.

Comparison of the usual Bachman precipitin test and the new microscopical test with living trichina larvae.

In 1928 *Bachman* described a precipitin test in trichinosis, using an acid hydrolyzed extract of trichina larvae as antigen. This test, which proved to be of great diagnostical value in clinical trichinosis, has been several times modified by other investigators. The present author obtained very satisfactory results with the following simplified modification of the test:

Trichina larvae, freed from their cysts in the manner described above and dried in a desiccator (with CaCl_2) at 37°C , were pulverized in an agate mortar and suspended in physiological saline in the ratio 1 g to 100 cc. The suspension was allowed to stand in the refrigerator for some days, being shaken up several times every day. After 5 days of extraction, the mixture was passed through a No. 3 Seitz filter. The clear filtrate which corresponds to an antigen dilution of 1:100 could be stored in the refrigerator for many months without any loss of antigenic power. At each experiment, further dilutions, viz. 1:500, 1:1,000, 1:2,000, 1:4,000 and sometimes also 1:8,000, were prepared of the original fluid. In each of the precipitin tubes of 5 mm bore, 0.2 cc of undiluted, unheated serum was overlaid with 0.2 cc of one of the antigen dilutions employed. The tubes were allowed to stand at room temperature (18°C). A reading for the formation of a white ring at the junction of serum and antigen was taken after 1 hour, then the tubes were shaken up and after 24

hours read again for flocculation, which gave much more clear-cut positive results than the ring formation.

The test was for the first time tried on 4 Swedish sera from persons who had suffered from trichinosis nearly a year before (*L. G. Hallén*, 1938)*). 2 of the sera were negative (the same sera had also proved to be negative, when they had been tested by Professor *Trawiński*, Lwów, in the acute phase of the infection). One serum was slightly positive with the 1:100 antigen dilution and the other strongly positive up to 1:2,000. The latter serum was found to be negative when tested again after another 6 months. On the other hand, all the 26 human sera (from abroad) mentioned above, which were tested more than 2 months after infection, gave positive reactions with the Bachman test, viz. 1 up to 1:1,000 antigen dilution, 16 up to 1:2,000 and 9 even up to 1:4,000, while 6 control sera were quite negative. In guinea pigs, the Bachman test was not so effective as the new microscopical test with living larvae. Of 12 guinea pigs, heavily infected by the ingestion of from 500 to 1,000 larvae, only 3 showed a definite reaction with the Bachman test (1 only with 1:100 antigen dilution, 2 up to 1:1,000). Of 8 rabbits, however, examined from the 14th to the 454th day after the ingestion of from 30,000 to 100,000 larvae, all were positive, in some cases even up to 1:8,000. But it is worth noting that one rabbit which was slightly positive with the microscopical test already on the 7th day, was at the same time still negative with the Bachman test.

Nevertheless, the two tests on the whole showed a fairly pronounced parallelism. Both seemed to be somewhat dependent on the severity of the infection in the strengths of their reactions, and both in the first weeks of the disease gave rather slight reactions which quickly increased up to a max-

*) The author wishes to thank Dr. *L. E. Hallén*, Lindesberg (Sweden), who was so kind as to provide the sera and give valuable information about the cases.

imum lasting from 2 months to about half a year after infection, then again very slowly decreasing.

The conclusion can be drawn, therefore, that both reactions may be due to the same antibodies being present in the immune serum. But, while the Bachman precipitin test, of course, is much easier to employ as a routine diagnostical test in clinical trichinosis, the more complicated, but apparently more delicate microscopical precipitin test with living trichina larvae might be an aid to the disclosure of early or other cases, where only a very slight antibody production has taken place.

Summary.

Living infective larvae of *Trichinella spiralis*, when incubated at 37° C in the sera of patients suffering from trichinosis or of guinea pigs or rabbits, experimentally trichinized, give rise to the formation in the sera of microscopically visible precipitates. However, no specific deleterious effect of such immune sera on the larvae has as yet been seen. The diagnostical value of this new microscopical test is compared with that of a simplified modification of the usual macroscopical Bachman precipitin test (using a filtrate of the saline extraction of dried trichina larvae as antigen). Both reactions seem to depend on the same antibodies present in immune serum, but the microscopical test may sometimes be more delicate in the demonstration of very slightly developed antibodies.

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ABSTRACTS — ANALYSES — REFERATE.

Die vitale Reaktion am Knochen. Von Gerhart Panning. (Veröffentlichungen aus der Konstitutions- und Wehrpathologie. Heft 45.) 116 Seiten, 27 Abbildungen, Preis brosch. 7,50 RM. Verlag von Gustav Fischer, Jena 1940.

Auf der Grundlage von Untersuchungen, die u. a. 3700 Schnittpräparate sowohl von intravitalen als auch von postmortalen Knochenbrüchen bei Menschen und Kaninehen umfassen, unterstreicht der Autor die auf jeden Fall dem Gerichtsmediziner nicht ganz unbekannte Tatsache, dass kleinere Blutungen an der Bruchstelle nach Eintreten des Todes entstehen können, und dass man nur bei grösseren Blutaustritten mit voller Sicherheit von einer vitalen Reaktion sprechen kann. Die Todesursache und die Blutfülle der Gewebe spielen natürlich eine bedeutende Rolle.

Eine Infiltration mit polymorphkernigen Leukozyten fand sich in einem Falle vor, in dem $2\frac{1}{3}$ Stunde vom Trauma bis zum Eintritt des Todes verflossen waren, doch meint der Autor, dass die leukozytäre Reaktion hier wie in anderen Geweben bereits $\frac{1}{2}$ Stunde nach der Läsion beginnt. Fettphagozytose fand sich ausnahmslos 20 Stunden nach dem Trauma, doch gelang es nicht, an dem vorliegenden Material festzustellen, wann der Prozess begann.

Im Experiment zeigte sich Weichteilsnekrose 65 Min. nach der Entstehung des Bruches, aber in dem einen Falle, in dem eine 1 Stunde alte Fraktur untersucht wurde, hatte es keine Weichteilsinterposition gegeben; das dürfte doch eigentlich nicht die Möglichkeit einer Weichteilsnekrose ausschliessen.

Die Arbeit, die ausschliesslich von Interesse für den Gerichtsmediziner ist, ist mit klaren und anschaulichen Mikrophotographien versehen.

V. Eskelund.

Karl-Heinz Kirschner: »Ueber den Status varicosus und die Bedeutung der Konstitution für die Entstehung der Varizen insbesondere im Pfortaderbereich; zugleich Bericht über eine eigenartige Form der Splenomegalie.« Veröff. a. d. Konstitutions- u. Wehrpathologie, Heft 44. Gustav Fischer, Jena, 1939. 148 S. Preis brosch. RM. 10.—.

Der Verfasser geht von dem Krankenblattauszug und Sektionsbericht über einen Fall von hochgradiger Varikose der Pfortader bei einem 23jährigen, bisher gesunden Manne aus, bei dem der Tod infolge einer Blutung aus Oesophagusvarizen eingetreten war. Die Rupturstelle ist deutlich auf der Photographie eines Oesophaguspräparates zu sehen, auf welchem die enormen Varizen mit blauer Gelatine injiziert worden waren. Die Sektion, die sehr gründlich durchgeführt worden war, wird ausführlich in allen ihren Details beschrieben. Der ganze Sektionsbefund in Verbindung mit dem jungen Alter des Patienten macht es wahrscheinlich, dass eine anlagemäss bedingte, krankhafte Venenverbildung vorgelegen hat, wie sie von der Konstitutionsforschung unter dem Begriff des »Status varicosus« in die moderne Pathologie eingeführt wurde und sinngemäss in der anatomischen Diagnose dieses Falles zum Ausdruck gekommen ist. Bei einer Untersuchung der Familie des Patienten fand der Verf., dass beide Eltern des Pat. sowie viele andere seiner Verwandten schwer varizenbehaftet waren.

Auf der Grundlage dieser Befunde und einer sehr gründlichen Literaturdurchsicht betont der Verf., dass sich sowohl klinisch als auch genealogisch der Nachweis dafür führen lässt, dass der Status varicosus als ein erbliches Leiden aufgefasst werden muss, welches auch das Pfortadersystem umfasst. Die Splenomegalie, die bei Status varicosus mit Lokalisation im Pfortadergebiet vorkommen kann, ist wahrscheinlich als konstitutionell anzusehen, und beide Anomalien sind auf die gleiche genetische Wurzel einer allgemeinen, im Mesenchym verankerten Bindegewebsschwäche zurückzuführen.

T. K.

STUDIES ON HEREDITARY DWARFISM IN MICE. IV.*)

ON THE FUNCTION OF METABOLIC ACTIVE HORMONES IN THE ANTERIOR PITUITARY DWARF MOUSE.**)

By *Carl J. Mollenbach****)

(Received for publication March 15th 1940).

The investigations, which are to be accounted for here, have been carried out on dwarf mice whose dwarf growth is due to a hereditary defect of the anterior lobe of the pituitary body. The rule of descent has been previously investigated by *G. D. Snell*, who found that the character was recessive and dependent on a single gene.

In a series of works *Philip Edw. Smith*, *MacDowell*, *T. Kemp*, *L. Marx* and others have illustrated various defects in the endocrine function of these animals. According to these investigators the defect of the anterior lobe of the pituitary body, which seems only to embrace eosinophil cells of the anterior lobe, must be considered to be the primary in relation to which other endocrine disturbances which have been discovered are secondary.

It is presumably justifiable to consider the animals with the hereditary defect of the anterior lobe of the pituitary body

*) Previous publications in this series:

Acta path. et microbiol. Scand. XIII, 1936, 512 og

» XIV, 1937, 197

» Suppl. XXXVII, 1938, 290

» XVIII, 1941, 20.

**) given as a lecture in slightly altered form to the Biol. Soc. Copenhagen d. 14. Feb. 1940.

***) The Author wishes to express his gratitude to Frk. P. A. Brandts Legat for a grant which made possible this investigation.

as partially hypophysectomized, and, it will, therefore, be of interest to review those metabolic anomalies which have been found as a result of the various forms of experimental hypophysectomy (isolated extirpation of the anterior lobe — total hypophysectomy etc.).

The first to show that there was a relationship between hypophysical ailments and metabolic anomalies was *Loeb*, who pointed out that glycosuria appeared with certain hypophyseal tumours.

Works by *Borchardt* (1908) and *H. Cushing* (1912) pointed especially towards the posterior lobe as being of importance for carbohydrate metabolism. Later, attention has been drawn, particularly by *B. A. Houssay* and his collaborators, to the fact that the function of the anterior lobe was of importance for metabolism.

In 1924 *Houssay* and *Magenta* showed that hypophysectomized dogs were extremely sensitive to insulin. One was, however, at that time most inclined to suppose that this was due to the removal of the posterior lobe.

E. M. K. Geiling's, *D. Campbell's* and *Y. Ishikawa's* works from 1927 supported this opinion.

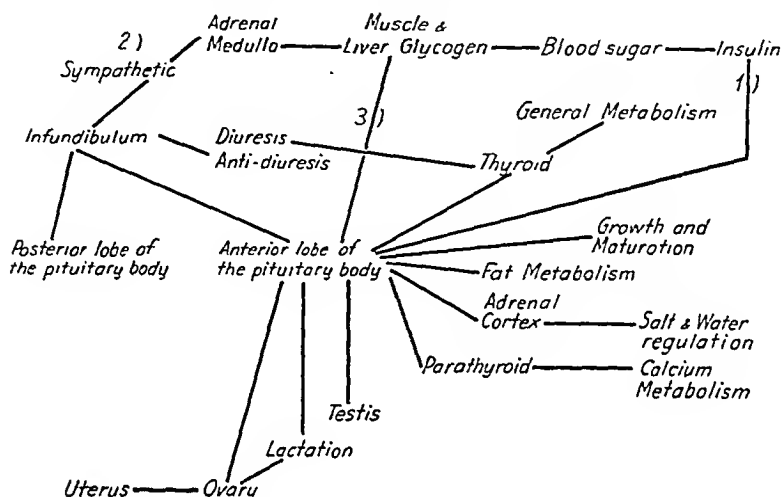
In 1929 *Houssay* and *Potick* showed that the anterior lobe of the pituitary body of toads can be removed without injury to the posterior lobe. These animals were found to be very sensitive to insulin. A daily dose of anterior lobe tissue counteracted this hypersensitivity while doses of pars intermedia or pars posterior were without effect. Similar results have later been achieved in mammals by a long line of authors — *Cori*, *Russel* and others.

It was concluded that, if the secretion of the anterior lobe was, amongst other things, of importance for the restricting or the hindering of insulin effect, i. e. it was antagonistic to insulin, then hypophysectomy would cause an improvement in a diabetic state. This was confirmed in 1930—1932 by *Houssay* and *Biasotti*.

In recent years a great number of papers referring to this hormonal regulation have appeared. A few only, representing the main trend of opinion, shall be mentioned here.

Following *Houssay's* assumption of a direct antagonistic effect of the secretion of the anterior lobe on insulin *Lucke* (1934) showed that hypophysectomy certainly improved a diabetic state, but the same effect could be achieved by the extirpation of the suprarenal glands. It was assumed that the secretion of the anterior lobe of the pituitary body operated via the mesencephalon — sympathicus — nervi splanchnici on the medullary part of the suprarenal gland, which, in turn, has a hormonal effect on the liver glycogen, so that this is mobilized as glucose.

O. Cope and H. P. Marks showed that hypophysectomized animals certainly released normal quantities of adrenalin after an insulin injection, but that the adrenalin was not capable of releasing the liver glycogen in the normal way and thus maintain the normal blood sugar values. The hyperglycemic effect of injected adrenalin



1) Houssay, Klin. Woch. 11, 1529. 1932.

2) Lucke, Ergeb. d. inn. Med. u. Kinderheil. 46, 94, 1934.

3) Cope & Marks: J. Physiol. 83, 157, 1934.

Fig. 1.

was also decreased. The thyroid function was found to be without influence on these conditions. It was considered that the secretion of the anterior lobe was necessary to render liver glycogen susceptible to suprarenal secretion (adrenalin).

Fig. 1 shows schematically the opinions mentioned with regard to the effect of metabolic active hormones.

Meanwhile other interpretations have also been proposed. Long and Lukens, for example, are of the opinion that the decisive factor is not the function of the medullary part of the suprarenal glands but of its cortex, in that diabetogenic extracts of the anterior lobe of the pituitary body are of no effect in cases where the cortex of the suprarenal glands is lacking.

These authors have produced a crystalline substance from suprarenal cortex which activates glucose formation from protein.

Other works exist besides those already mentioned. Anselmino, for instance, who, partly in co-operation with Hoffmann, has brought

important arguments to bear on the discussion on metabolic active hormones. It should be mentioned that he accepts both the counter insulinary principle described by *Lucke* and also the diabetogenic hormone found by *Houssay*, in that he maintains that their functions are different, amongst other reasons also because the anti-insulinary hormone has a far quicker effect after injection than was described by *Houssay*.

Anselmino mentions a couple of hormones having effect on carbohydrate metabolism, — the corticotropic anterior lobe hormone and that hormone which has been described by *Effkemann* (1935), the carbohydrate metabolism hormone which, in the course of a very short time, is capable of emptying all glycogen depots.

It is *Anselmino* too who has described the so-called ketogenic hormone of the anterior lobe which is without effect on carbohydrate metabolism.

That the diabetogenic »hormone« does not bear its name without cause has been proved by *F. G. Young* (1937). After an injection treatment over a considerable period he produced in dogs a veritable diabetic state which remained for years after the hormone injections had ceased. Postmortem examination of these dogs revealed pronounced degeneration of the cells of the islets of Langerhans. So the term »hormone« can not be said to be adequate.

A number of investigations regarding the diabetogenic hormone have also been carried out in Denmark. *T. Bjerling*, amongst others, has shown that it is to be found in great quantities in the urine of pregnant women and in that of certain diabetics where the ketogenic hormone (the fat metabolism hormone) and the carbohydrate metabolism hormone are also present.

The results are remarkable in that abundant quantities of eosinophil cells are to be found in the anterior lobe of pregnant women. Corresponding conditions have been found in acromegals, but hardly in diabetics.

There thus exists a great material concerning investigations of various grades of failing function of the anterior lobe and concerning certain forms of partial hyperfunction (the various types of chromophile adenomata, increase of the eosinophil cells in the anterior lobe under certain conditions).

On the other hand, however, I have found no works on spontaneous partial hypofunctions in animals apart from the former investigations on that mouse strain which I have employed in my experiments.

As has been mentioned a defect of the anterior lobe of the

pituitary body is present in a number of the mice in this strain, and this has been proved to be due to an elective, almost total lack of eosinophil cells.

All the individuals of the strain which are struck by the defect become dwarfs and differ in many respects from the normal. The appearance and physiology of these dwarfs can shortly be described as follows:

The dwarf growth is first apparent in the second week. The dwarfs remain small and their weight hardly exceeds $\frac{1}{4}$ — $\frac{1}{3}$ of that of their normal brothers and sisters. The shape of the head is broader than is normal. The nose is short and blunt, the ears small and rounded.

The posture differs from the normal, the dwarfs being more crouching, short, almost spherical in appearance, in contrast to normal mice which are rather long and slim.

The gait is slow and stiff. The coat not smooth, thick and glossy as in normal mice, but more ragged, thinner and almost dull.

Metabolism is considerably reduced — to c. 60 % of the normal.

No information is at hand as to the functioning of the heart, liver, kidneys, lungs or digestive tract. The mice eat well, and are able, when they are about a month old, to manage without their mother provided the temperature is suitable; they are very sensitive to cold and succumb easily if the temperature in the cage drops below c. 20—22° C.

The dwarfs are, moreover, sterile; their sexual organs are not absolutely infantile but the testes and ovaries only attain a certain slight degree of development. Castration produces distinct changes in the secondary sexual characteristics.

Former investigators have pointed out considerable changes in the endocrine organs. *Smith* and *MacDowell* have, for instance, shown that the anterior lobe of the pituitary body is hypoplastic with complete or almost complete lack of eosinophil cells.

Later investigations by *T. Kemp* and *L. Marx* (1936—37) showed: The thymus comparatively small; the thyroid gland presented distinct and in part very widespread fatty degeneration of the epithelium; no typical differentiation of the cell strings was found in the parathyroid gland, the cells being small with a slight amount of protoplasm; the testes and ovaries only slightly developed, almost infantile; the medullary part of the suprarenal gland normal; the cortex somewhat small, zona fasciculata was especially small and zona reticularis especially defective in development; the pancreas seemed normal in young dwarfs but was found to be pronouncedly atrophied in the elder. An exact investigation of the amount of the

tissues in the islets of Langerhans in normal mice and in dwarfs of different ages has, however, not yet been published.

Own investigations:

As, by reason of the nature of the defect of the anterior lobe of the pituitary body, it could be presumed that these dwarfs presented anomalies in their carbohydrate and fat metabolism, and, as there did not exist any investigations in this matter, I decided to examine certain aspects of their metabolic functions by:

- 1) Microscopic examination of the fat and glycogen content of the liver cells after feeding on a standard diet.
- 2) Microscopic examination of the fat and glycogen content of the liver cells in animals fed on a standard diet and then starved for a week.
- 3) Examination of the sensitiveness of dwarfs and normal mice to insulin.

So as to have as good an animal material as possible I have, in all the experiments, only used dwarfs having normal brothers and sisters of the same sex which were used as controls. Animals ranging in age from 14 days to a good 6 months with a difference in age of 2 weeks have been examined. The examination has embraced both males and females but no difference in the deposits in the liver cells conditional upon sex was apparent. The above described choice of material has entailed the stretching of the experiments over a number of months.

As the diet is of especial importance for liver deposits it must be stated that the diet the animals received was in every way sufficient. Two diet forms were used:

- 1) Alternately salad, spinach, corn, rye bread and cod liver oil, milk and white bread together with water.
- 2) Cooley's Standard feed: dried milk 490 g., casein 735g., vegetable fat 245 g., Lucerne meal 145 g., ground wheat 3.320 g., cooking salt 50 g., chalk precipitate 75 g., cod liver oil 12 ccm., water 6 l.

A change over from the one diet to the other was made after it had been proved by control experiment that the liver cell deposits did not change in character.

Group 1.

Examination of the fat and glycogen content of the liver cells in animals on standard feed. All the animals were killed at the same time of day (10—11 a.m.). No preliminary starvation period.

*Postmortem results:**Normal Mice:*

Vigorous; good musculature; abundant fat deposits. Liver: large, pale, yellowy-brown.

Dwarf Mice:

Small; musculature fairly well developed; slight fat deposits. Liver: small, darker, reddish-brown.

*Microscopic examination:**Normal Mice:*

In celloidin section stained according to Best abundant glycogen was found in the cells.

In freezing section stained with Sudan extremely abundant fat deposits were to be seen. The fat occurred in all parts of the lobules; the fat granules in the liver cells varied greatly in size, from some fine as dust to others larger than the nucleus. No degenerative changes.

Dwarf Mice:

In colloidin section stained according to Best very abundant glycogen was found in the cells, far more than in the normal. The deposits were of the greatest intensity in the central parts of the lobules and round about venae sublobulares. In freezing section stained with Sudan only rather scanty fat was found. It was deposited especially in the intermediary and peripheral parts of the lobules.

There was thus a distinct difference in the fat and glycogen deposits in the livers of normal mice and dwarfs; the difference was, however, still more marked when the animals had fasted for a time.

Group 2.

Examination of the deposits in the liver cells of animals which have been starved for 24 hours after having been fed on a meagre diet i.e. scant bread and water 1 week. The animals were killed as in group 1.

The autopsy showed macroscopic conditions as in group 1.

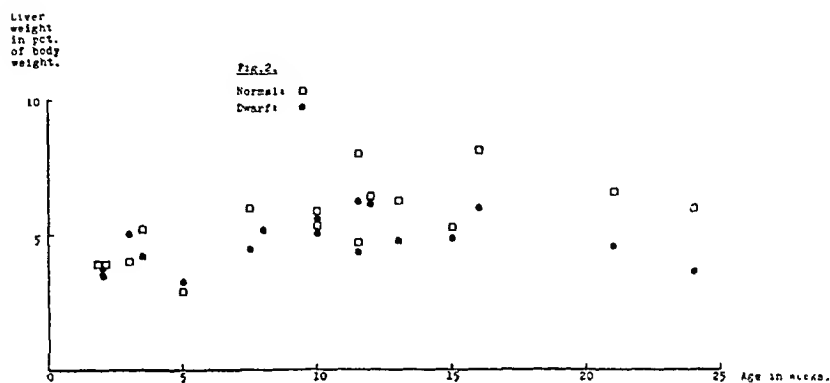
Table 1.

Experi- ment Nr.	Age in weeks	D = Dwarf N = Normal	Body weight in g.	Liver weight in g.	Liver weight in pct. of body weight
43	2	D	5	0,175	3,5
45	2	N	5,1	0,200	3,9
44	2	D	5	0,185	3,7
46	2	N	5	0,205	3,9
15	3	D	4,2	0,210	5,0
16	3	N	8,8	0,350	4,0
13	3,5	D	5,5	0,23	4,2
14	3,5	N	9,3	0,48	5,2
27	5	D	2,5	0,08	3,2
28	5	N	5,5	0,16	2,9
11	7,5	D	4	0,180	4,5
12	7,5	N	15,2	0,920	6,0
63	8	D	4,8	0,25	5,2
19	10	D	6,75	0,375	5,5
20	10	N	15,5	0,850	5,4
5	10	D	8	0,407	5,1
6	10	N	26,5	1,560	5,9
1	11,5	D	5	0,315	6,3
2	11,5	N	12,5	1,005	8,0
3	11,5	D	4,5	0,200	4,4
4	11,5	N	12,5	0,597	4,7
21	12	D	5,85	0,365	6,2
22	12	N	22,5	1,450	6,4
25	13	D	4,76	0,23	4,8
26	13	N	25,7	1,64	6,3
7	15	D	5,5	0,286	4,9
8	15	N	21,5	1,150	5,3
9	16	D	5,25	0,320	6,0
10	16	N	20,0	1,625	8,1
23	21	D	5,69	0,26	4,6
24	21	N	22,8	1,53	6,6
17	24	D	7,65	0,28	3,7
18	24	N	23,4	1,42	6,0

Weighing of the extracted livers revealed, as did the weight of the animals, great variation. The liver weight expressed in pct. of the body weight varied from c. 3—8 %. There was, however, as appears in table 1 and fig. 2 a pronounced ten-

dency for the liver weight of the normal mice, also expressed in pct. of their body weight, to be considerably greater than the corresponding weight proportions of the dwarfs.

As especial interest is attached to this experimental group



the results of the microscopic examination of each individual animal in this group are reproduced in table 2.

Microscopic examination with regard to the fat content of the liver.

The examination was conducted on 5—7 μ thick freezing section. The fat was stained with Sudan and the nuclei with hematoxylin. All the organs examined revealed normal tissue structure, although it must be remarked that the dwarf livers give the impression of having a somewhat inferior degree of tissue differentiation to the normal (see table 2). This is probably connected with the lesser size of the dwarf organs.

Microscopic examination with regard to the glycogen content of the liver.

The examination was conducted on a c. 10 μ thick celloidin section. The nuclei were stained with hematoxylin and the glycogen according to Best's method (see table 3).

Microscopic examination with regard to the glycogen content of the liver cells.

Table 3.

Experiment Nr.	D = Dwarf N = Normal	Age in weeks	Glycogen in Lobules			Glycogen in cells round about vv. sublobulares
			centrally	inter- medially	peripherally	
43	D	2	+++	++	++	+
45	N	2	+	+	+	(+)
44	D	2	+++	++	++	(+)
46	N	2	+	+	+	(+)
15	D	3	++	++	+	++
16	N	3	(+)	(+)	÷	÷
13	D	3,5	+++	+++	+++	+++
14	N	3,5	+	++	+	÷
27	D	5	+	++	++	+
28	N	5	÷	÷	÷	÷
11	D	7,5	++	+	+	+
12	N	7,5	+	+	÷	+
63	D	8	++	++	+	+
19	D	10	+	+	(+)	÷
20	N	10	÷	÷	÷	÷
5	D	10	+++	++	+	++
6	N	10	÷	÷	÷	÷
1	D	11,5	+	++	+	(+)
2	N	11,5	(+)	÷	÷	÷
3	D	11,5	+++	++	+	++
4	N	11,5	÷	÷	÷	÷
21	D	12	++	+	+	+
22	N	12	÷	÷	÷	÷
25	D	13	+	+	+	(+)
26	N	13	÷	÷	÷	÷
7	D	15	+++	+++	++	++
8	N	15	÷	÷	÷	÷
9	D	16	++	+	+	+
10	N	16	÷	÷	÷	÷
23	D	21	+	++	+	÷
24	N	21	÷	÷	÷	÷
17	D	24	+	+	+	+
18	N	24	÷	÷	÷	÷

÷ no glycogen
(+) scant glycogen
+ some glycogen

++ abundant glycogen
+++ very abundant glycogen

To illustrate the metabolic function of these mice I have, furthermore, in collaboration with *E. Bartels*, examined their resistance against insulin.

Owing to the small size of the dwarf mice it is not possible to obtain sufficient blood for a blood sugar determination without killing the animal, in which case sufficient blood for one determination only can be obtained, and sometimes one must even be content with a half portion blood to make the determination, which does not increase its accuracy.

We have, therefore, elected to use the appearance of hypoglycemic cramps as a test.

In our preliminary experiments we found that, after having been starved for 24 hours our normal mice got cramps about a couple of hours after receiving insulin doses which lay about 8/1000 I. E. insulin pr. 3,5 g. body weight.

We then selected pairs of brothers and sisters (dwarf + normal mouse of the same sex) of different ages which, after 24 hours starvation, were given a dose of insulin proportionate to their weight, after which the cages containing the mice so treated were placed in a temperature of c. 25° C.

It appears that quite young mice — both dwarf and normal — have a relatively high insulin tolerance. Where the animals have attained an age of c. 4—5 weeks the dwarfs show throughout a far greater insulin sensitiveness than the normal, in that the dwarfs most frequently get hypoglycemic cramps before an hour has elapsed, while the normal mice do not usually get cramps even after the course of 2 hours.

The results appear in table 4 I and II and in the graphic presentation fig. 3. It will be seen that a few normal mice are somewhat insulin sensitive, but, in these cases it will also be found that the corresponding dwarfs show an even greater sensitiveness to the treatment.

The investigations have thus shown that, between normal mice and dwarf mice which are kept on an ordinary good diet, there is a distinct difference in the amount of glycogen and fat deposited in the liver cells, in that livers from the normal mice contain abundant quantities of both fat and glycogen,

Table 4. — I.

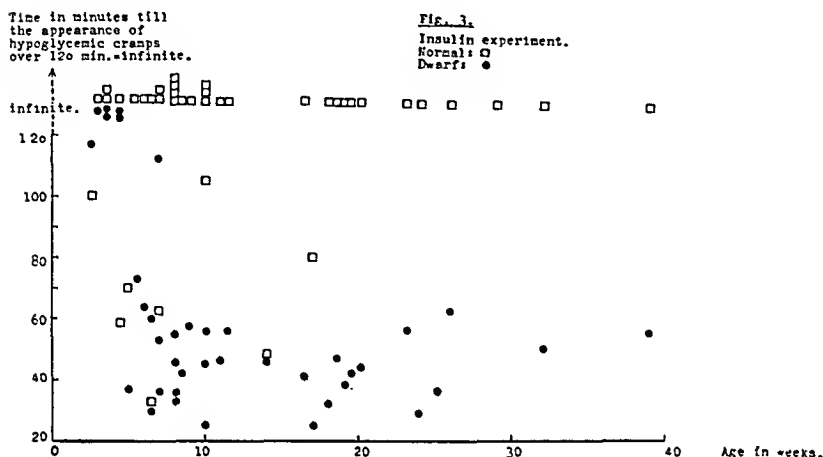
Experi- ment Nr.	Age in weeks	D = Dwarf N = Normal	Sex.	Body weight in g.	Insulin dose in $\frac{1}{10}$ cc. à $\frac{8}{1000}$ I. E.	Time in minutes till the appea- rance of hypo- glycémie cramps
27	2,5	D	♀	5,5	1,5	117
	—	N	♀	6,0	1,5	100
24	3	D	♂	6,0	2,0	> 120
	—	N	♂	11,0	4,0	> 120
10	3,5	D		4,5	1,5	> 120
	—	N		9,5	3,0	> 120
11	3,5	D		6,0	2,0	> 120
	—	N		9,0	3,0	> 120
19	4,5	D	♂	6,5	2,0	127
	—	N	♂	12,0	4,0	59
20	4,5	D	♂	4,0	1,0	> 120
	—	N	♂	11,0	3,0	> 120
13	5	D	♀	6,5	2,0	37
	—	N	♂	17,0	5,0	70
26	5,5	D	♂	7,0	2,0	73
	—	N	♂	19,5	5,0	> 120
15	6,5	D		6,0	2,0	30
	—	N		21,5	7,0	34
9	6	D		6,0	2,0	64
	—	N		19,5	6,0	> 120
8	6,5	D		4,5	1,5	60
	—	N		17,5	5,0	> 120
7	7	D		6,5	2,0	53
	—	N		22,0	6,0	63
17	7	D	♀	7,0	2,0	36
	—	N	♂	30,0	8,0	> 120
31	7	D	♂	8,0	3,0	113
	—	N	♂	18,5	6,0	> 120
38	8	D	♂	7,5	2,5	33
	—	N	♂	23,0	8,0	> 120
25	8	D	♂	7,5	2,0	46
	—	N	♀	21,0	6,0	> 120
21	8	D	♂	5,5	1,5	55
	—	N	♂	20,0	6,0	> 120
6	8	D		4,0	1,5	35
	—	N		19,0	5,0	> 120
18	8,5	D	♂	7,0	2,0	42
	—	N	♂	25,0	7,0	> 120

Table 4. — II.

Experi- ment Nr.	Age in weeks	D = Dwarf N = Normal	Sex.	Body weight in g.	Insulin dose in $\frac{1}{10}$ cc. $\frac{1}{1000}$ I. E.	Time in minutes till the appea- rance of hypo- glycemic cramps
23	9	D	♀	5,0	1,5	57
	—	N	♂	22,0	6,0	> 120
29	10	D	♂	8,0	3,0	45
	—	N	♂	26,0	8,5	> 120
32	10	D	♂	7,0	2,5	56
	—	N	♂	27,0	9,0	106
35	10	D	♂	8,5	3,0	25
	—	N	♀	24,0	8,0	> 120
30	11	D	♀	8,0	3,0	46
	—	N	♀	27,0	9,0	> 120
34	11,5	D	♀	6,0	2,0	56
	—	N	♂	24,5	8,0	> 120
5	14	D		8,0	2,0	47
	—	N		26,5	7,0	48
22	16,5	D	♀	8,0	2,0	41
	—	N	♂	29,0	8,0	> 120
12	17	D		7,5	2,0	25
	—	N		15,0	4,0	80
4	18	D		8,0	2,0	32
	—	N		19,5	5,0	> 120
3	18,5	D		7,5	2,0	47
	—	N		26,0	7,0	> 120
33	19	D	♂	9,5	3,0	38
	—	N	♂	31,0	10,0	> 120
28	19,5	D	♂	8,0	2,0	42
	—	N	♂	21,0	6,0	> 120
39	20	D	♀	7,0	2,0	44
	—	N	♂	27,5	7,0	> 120
36	23,5	D	♀	7,5	2,5	57
	—	N	♂	30,0	10,0	> 120
37	24	D	♂	9,0	3,0	29
	—	N	♂	24,5	8,0	> 120
16	25	D		7,5	2,0	36
2	26	D		8,5	2,0	63
	—	N		39,0	10,0	> 120
1	39	D		7,5	2,0	55
	—	N		33,0	9,0	> 120
14	32	D		7,0	2,0	50

but the dwarfs' livers do not contain great quantities of fat, as the dwarfs seem to have difficulty in depositing fat at all while, on the other hand, the glycogen content of the liver is great.

If the animals are starved it is found that the glycogen



in the normal mice is quickly and almost completely mobilized, contrary to the fat which only slightly diminishes.

The dwarfs seem to be capable of readily mobilizing the little fat they are able to deposit, but they are not capable of mobilizing the glycogen depots in the normal way.

The dwarfs are far more sensitive to insulin than are normal mice.

If the experimental results in the literature mentioned above be compared with the experimental results of the investigations of dwarf mice: abundant liver glycogen, diminishing with age and readily mobilizable; scant fat content; great insulin sensitiveness etc. it will seem that the experiments support the opinion that the former proven lack of eosinophil cells in the anterior lobe of the pituitary body in dwarfs not only causes the partial lapse of the growth hormone, the gonadotropic, the thyrotropic and the parathyrotropic hormones of the anterior lobe, but it also causes a

deficient secretion of one and perhaps of several metabolic active hormones which influence carbohydrate metabolism and fat metabolism.

Summary.

Microscopic investigations with the object of examining the fat and glycogen content of the liver cells, and especially the mobilization of these substances during fasting have been carried out on mice belonging to a strain in which appears an hereditary dwarf growth due to a complete or partial lack of eosinophil cells in the anterior lobe of the pituitary body. Organs from both normal and dwarf mice have been investigated, partly after feeding with Cooley's standard feed and partly after a fasting period. The fat deposits in the dwarfs were found to be very scanty in all cases. After Cooley's standard feed it was found that the liver of the dwarf mice contained very abundant quantities of glycogen more than that of the normal control animals; the fat content on the other hand was scanty in the dwarfs whilst the normal mice had very abundant quantities of fat in their livers.

If the animals were starved it was found that the glycogen in the normal mice was rapidly and almost entirely mobilized contrary to the fat which only diminished slightly. The dwarfs seemed to be able to mobilize easily that little fat they were capable of depositing, but they were not able to mobilize the glycogen deposits in the normal way.

The dwarf mice were more sensitive to insulin than the normal ones. It was supposed that the lack of eosinophil cells in the anterior lobe of the pituitary body caused a hyposecretion of one or more metabolic active hormones.

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UNTERSUCHUNGEN ÜBER CATGUT. I. VERGLEICHENDE VERSUCHE ÜBER DIE RESORP- TIONSZEIT IN RATTENMUSKULATUR.

Von *Henry M. Christensen* und *Poul V. Marcussen*.

(Eingegangen bei der Redaktion am 30. April 1940).

Wenn die moderne Chirurgie immer noch Catgut für versenkte Suturen verwendet, beruht dies ausschliesslich auf der Resorbierbarkeit dieses Materials. Diese Eigenschaft, die bereits im Altertum bekannt war (vgl. die umfassende Übersicht von *Bullock, Lampitt & Bushill*), kann indessen nur ausgenutzt werden, wenn zwei Hindernisse überwunden werden: 1) Die technische Schwierigkeit der Verarbeitung des organischen Gewebes (Schafdarm) zu einem Faden von ausreichender Festigkeit und Geschmeidigkeit im Verhältnis zur Dicke; 2) Die Schwierigkeit einer effektiven Sterilisation des Darmgewebes, das fast immer sehr resistente Bakteriensporen enthält, und das selbst von den meisten bakterientötenden Prozessen stark beeinflusst wird. Ein verwendbares Catgut muss daher in drei Beziehungen gewisse Forderungen erfüllen können: Mechanische Eigenschaften, Sterilität und Resorbierbarkeit.

Die technische Bearbeitung des Schafdarmes ist ein altes Handwerk, dessen Methoden bekannt und erprobt sind. Das Problem der Catgutherstellung liegt zur Zeit in der Forderung nach absoluter Sterilität, wie dies in den letzten Jahren besonders von amerikanischen Verfassern scharf hervorgehoben wurde (*Meleney & Chatfield, Clock, Dalrymple-Champneys, Brewer*).

Wenn auch die chemischen Sterilisationsmethoden bei einer absolut korrekten Durchführung gute Resultate ergeben können, so geht die Tendenz doch mehr in der Richtung der physikalischen Methoden, da es sich erwiesen hat, dass ein sehr grosser Prozentsatz der chemisch behandelten Präparate nicht zuverlässig steril war (*Clock*). Die Wärmesterilisierung enthält wie jede Methode die Gefahr, andere wichtige Eigenschaften des Fadens wie Zerreiissfestigkeit und Resorbierbarkeit zu beeinflussen. Während die Zerreiissfestigkeit und Geschmeidigkeit des Fadens einer exakten Beurteilung leicht zugänglich sind, ist es schwierig, auf die Haupteigenschaft des Catgutfadens, die Resorbierbarkeit, Schlüsse zu ziehen.

Bei Durchgang der Literatur finden wir eine bedeutende Anzahl Methoden, die in der Hauptsache in drei Gruppen geteilt werden können:

- 1) Bestimmung der Tensionsstärke (oder Zerreiisspunkt) *in vitro*.
 - 2) Bestimmung der Tensionsstärke (oder Zerreiisspunkt) *in vivo*.
 - 3) Histologische Beurteilung des Resorptionsverlaufes im Tierversuch.
-

ad 1. Durch Digestionsversuche ist die Wirkung einer einfachen Erweichung des Fadens untersucht worden. *Mayer* fand, dass diese zu keiner vollständigen Auflösung des Fadens führte, wies aber nach, dass Leimwasser den Faden in mehrere erweichte Teile zerlegte. Serum wirkte bei 30°—40° sehr langsam auf Catgut, und Galle konnte den Faden im Laufe von 18 Monaten nicht auflösen. *Brunner* zeigte, welche Bedeutung die Anwesenheit von Leukozyten hatte. Beim Stehenlassen in Eiter wurde der Faden im Laufe von 9 Tagen total destruiert, wogegen eine sichtbare Beeinflussung bei Stehenlassen in Serum allein oder in Serum mit einer Bakterienkultur während 42 Tagen nicht festzustellen war.

Diese Versuche sind 1928 von *Howes* wiederholt und er-

weitert worden. Es wurde die Tensionsstärke des Fadens als Test verwendet, und die Untersuchungen zeigten den Unterschied in der Abnahme der Tensionsstärke, wenn der Catgutfaden von frischem Serum, infiziertem Serum, Aleuronatexsudat und physiologischer Salzlösung beeinflusst wurde. Die Tensionsstärke ging im Serum etwas zurück, schneller im Aleuronatexsudat, aber am schnellsten im infizierten Serum. Auch in physiologischer Salzlösung wurde eine recht grosse Abnahme der Tensionsstärke festgestellt. Die Versuche zeigten somit, dass der Faden einen Teil seiner Festigkeit beim Erweichen in den verwendeten Flüssigkeiten verlor, und dass das Vorhandensein von Bakterien die Auflösung begünstigte, was mit den Ergebnissen der Tierversuche von *Howes* u. a. übereinstimmte. Die Versuche mit Aleuronatexsudat wurden mit täglicher Erneuerung des Exsudats wiederholt. Hierdurch wurde die Schwächung der Zerreiissfestigkeit des Fadens merkbar beschleunigt.

In einer Anzahl von Versuchsreihen wird Digestion mit proteolytischen Enzymen angewandt. So benutzte *Reil* eine Pepsin-Ameisensäurelösung.

Kraissl & Meleney haben eine ähnliche Pepsinmethode angewandt. Die Untersuchungen zeigten, dass die Zerreiissfestigkeit bei Catgutfäden von zehn verschiedenen Herstellerfirmen während der Digestion in verschiedenem Grade abnahm, während sie bei verschiedenen Fäden von derselben Firma in gleicher Weise abnahm, ein Verhalten, das sich mit den Erfahrungen vergleichen lässt, die man in bezug auf die Wirkung verschiedener Stoffe auf Catgut während der chemischen Sterilisation gemacht hat. Catgut, das durch Kochen sterilisiert wird, zeigte sich weniger resistent als anderes Catgut; nicht-sterilisiertes Catgut wurde am langsamsten beeinflusst, eine Erfahrung, die von verschiedenen Untersuchern gemacht wurde.

Die Digestionsversuche geben, was auch von *Kraissl & Meleney* hervorgehoben wird, keinen Ausdruck für die Resorptionszeit.

Mit Versuchen *in vitro* können die chemischen Verhält-

nisse im Organismus nur unvollständig, und die Wirksamkeit der lebenden Zelle überhaupt nicht reproduziert werden. Die letztere aber muss, wie nach dem histologischen Bild anzunehmen ist, eine entscheidende Rolle im Resorptionsprozess spielen.

ad 2. Einen sehr interessanten Eindruck von der Variation der Tensionsstärke während der Resorption geben Versuche an Menschen und Tieren, bei welchen die Veränderungen in der Zerreißfestigkeit des Fadens, während er unter Einwirkung des lebenden Gewebes steht, gemessen wurden.

Callender plazierte eine Anzahl karbolbehandelter Catgutfäden in einer Operationswunde eines Patienten so, dass die Mitte der Fäden unter Einwirkung des Wundsekretes war. Nach 24 Stunden begann der Umfang der Fäden abzunehmen, und nach 55—60 Stunden war die Mittelpartie sämtlicher Fäden verschwunden. Güterbock zeigte, dass Catgut, das in der Achillessehne von Kaninchen und weissen Ratten eingenäht war, noch nach 14 Tagen im Gewebe nachgewiesen werden konnte. Die Festigkeit war gradweise abnehmend. Macewen benutzte zu seinen Versuchen Operationspatienten, indem er in 31 Fällen eine Chromcatgutsutur tief im Gewebe anlegte, so dass die Enden des Fadens frei aus der Haut herausreichten. Als Zeitpunkt, an dem die Fäden durch Ziehen an den Enden zum Zerreißen gebracht werden konnten, wurde der 9.—19. Tag bestimmt. In einer anderen Versuchsreihe wurde gestossen wurden, auf den 15.—24. Tag bestimmt. Vermutlich war der Faden zu diesem Zeitpunkt resorbiert. Booth untersuchte die Zerreißfestigkeit von Catgut, das nach verschiedenen Methoden sterilisiert war, 1—6 Tage nach Einnähen in der Muskulatur von Hunden und fand eine bedeutende Abnahme in der Festigkeit des nicht-chrombehandelten Catguts, wogegen Chromcatgut nur unbedeutend an Festigkeit verlor. Die Versuchszeit war, wie erwähnt, sehr kurz. Die von Howes ebenfalls an Hunden ausgeführten Versuche zeigten in

gleicher Weise eine bedeutende Abnahme der Festigkeit des Fadens im Laufe von 5 Tagen, am meisten bei dünnen Fäden.

Wolff & Priestley prüften 358 Catgutfäden an 164 Patienten mittels einer Technik, die an die von *Macewen* erinnert, jedoch mit der Modifikation, dass an den Catgutfaden ein unresorbierbarer Faden befestigt wurde, der aus der Haut herausreichte. Der Zeitpunkt, an welchem die Fäden manuellem Zug nicht mehr widerstehen konnten, war bei Chromcatgut der 10.—20. Tag, bei »plain« Catgut der 8.—14. Tag. Doppelte Suturen wurden ebenso schnell resorbiert wie einzelne. Chromcatgut Nr. 000 wurde in 15 Tagen resorbiert, während Nr. 1 und 2 in 10,6 Tagen resorbiert wurden. Bei Versuchen nach dieser Methode ist nachgewiesen worden, dass die verschiedenen Catgutarten durchschnittlich solange in den Geweben liegen bleiben, bis die Wunde eine ausreichende Stärke erreicht hat. So wiesen *Howes, Sooy & Harvey* bei Versuchen an Hunden nach, dass eine Faszienwunde ihre volle Stärke ca. am 14. Tag, eine Muskelwunde ca. am 12. Tag und eine Magenwunde ca. am 12. Tag erreichte. Aber bereits nach Verlauf der halben Zeit war die Stärke der Wunden im schnellen Zunehmen.

Die späteren Versuche von *Howes* (1933) bestanden in der Suturen von Wunden im Rattenmagen mit dünnem Catgut, Chromcatgut und Seide und nachfolgenden Serienversuchen mit Ausweitung des Magens bis zum Zerreißen der Wunden. Alle Wunden hatten die gleiche Stärke bei Beginn der Heilung. Danach nahm die Stärke ab für »Catgutwunden« bis zum 4. Tag, für »Seidenwunden« bis zum 3. Tag. Hierauf nahm die Stärke schnell zu, indem »Seidenwunden« bis zum 7. Tag widerstandsfähiger waren. Dann waren beide Wundarten gleich stark und widerstanden der Ausweitung bald besser als das umliegende Gewebe.

Von diesen Methoden ist somit keine zum Vergleich von Catgutarten auf breiter Basis geeignet.

ad 3. Das Prinzip bei den histologischen Methoden ist, die Zeitpunkte für das Verschwinden des Fadens und seine Er-

stattung durch Bindegewebe bei Versuchstieren zu bestimmen. In dieser Gruppe liegen eine sehr grosse Anzahl von Untersuchungen vor. Bereits vor Einführung der Antiseptik gab *Porta* (1845) eine gründliche Beschreibung des Verhaltens des Fadens im Organismus, u. a. der adhäsiven Entzündung, bei welcher der Faden von einem zellhaltigen Exsudat bedeckt wird, wonach er sich aufrollt, aufgeweicht und mit dem Gewebe »vermischt« wird.

Lister, der die versenkte sterile Suture in die Chirurgie einführte, hat besonders die »Organisation« des Catguts beschrieben, d. h. die Substitution zuerst mit einem weichen Bindegewebe bestehend aus Fibroblasten und mehrkernigen Zellen, später mit einem fest organisierten Bindegewebe, das etwa am 14. Tage in Erscheinung tritt.

Die Bedeutung des Granulationsgewebes für die Zersetzung des Fadens wurde von *Eliaschewitch* beobachtet. *Lister* und später *Cheyne* wiesen nach, dass der präparierte Catgutfaden von der Oberfläche aus angegriffen wird und erst später in der Tiefe. *Hallwachs* wies nach, dass Catgut bei Hunden im Laufe von 6 Monaten vollständig resorbiert wurde.

Hinsichtlich der Wahl der Versuchstiere sind interessant die Kaninchenversuche *Lesser's* (1884), welche zeigten, dass Catgut in der Muskulatur dieser Tiere 64—85 Tage unbeeinflusst war. Diese Beobachtung wird durch einen Teil der Versuche in der bahnbrechenden Arbeit von *Claudius* (1906) bestätigt, bei denen Catgut im Peritoneum von Kaninchen noch 126 Tage nach der Einlage nachgewiesen wurde.

Porrit, der ebenfalls Kaninchen verwendete, wies nach, dass Seide eine stärker ausgeprägte Fremdkörperreaktion veranlasste als Catgut.

Lesser hebt ebenso wie *Booth*, *Ilyin* u. a. die Bedeutung hervor, die sowohl die Beschaffenheit des Fadens wie auch die Art des Gewebes hat. *Minervini* zeigte, dass chemisch präpariertes Catgut langsamer resorbiert wurde als wärmesterilisiertes. Chromcatgut wurde besonders langsam resorbiert. Zu einem ähnlichen Resultat kam *Claudius*, indem er die Resorptionsverhältnisse für Jodcatgut besonders günstig fand.

Fleming meinte, dass Entzündung die Resorption hindert, eine Ansicht, die von *Claudius*, *Fürle* und *Braun* geteilt wird; letzterer gibt jedoch an, dass Catgut, das zuerst in einem Abszess gelegen hat, seine Oberfläche eingebüsst hat und bei Einlage in die Subkutis eines Kaninchens leicht resorbiert wird. Einzelne Forscher kamen zu einem entgegengesetzten Ergebnis, wie z. B. *v. Bruns* und *Brunner*, die meinen, dass Catgut in suppurierenden Wunden schneller verschwindet.

Serienversuche mit gleichartiger Technik sind von *Goris & Rolland* ausgeführt worden, die mit einer Reverdinnadel Catgut in die Schenkelmuskulatur von Meerschweinchen nach chirurgischer Präparation der Haut einnähten. Bei Untersuchung von nach verschiedenen Methoden sterilisiertem Catgut zeigte es sich, dass die meisten Arten noch nach 48 Tagen unresorbiert waren. Die Verfasser massen der physikalischen Beschaffenheit des Fadens grössere Bedeutung zu als der chemischen.

Christ wies nach, dass die Gewebe, wo die Untersuchung der Fadenresorption am wichtigsten und histologisch am leichtesten zu verfolgen sei, Muskel und Faszie sind. *Ziegler & Clark* benutzten die Röntgenuntersuchung von Catgut vor und während des Sterilisationsprozesses sowie während der Resorption und gaben an, dass sie in der Lage seien, Catgut nach Stärke und Resorbierbarkeit auszuwählen. *Bates* zeigte durch Versuche an Hunden, dass »plain« Catgut schnell eine starke Reaktion gab, die das Wachstum von Fibroblasten hinderte und die Heilung der Wunde verzögerte. Dicke und dünne unbehandelte Catgutfäden wurden gleich schnell, und zwar zu schnell, resorbiert. Chromcatgut gab eine geringere Reaktion, also frühere Heilung. Dünne Chromcatgutfäden wurden langsamer resorbiert als dicke und unterstützten daher die Wundheilung am besten.

Aus der Literatur geht somit hervor, dass die Bestimmung der Tensionsstärke in vivo in Relation zum Vernarbungspro-

zess sehr interessant ist, dass aber die Methode sich kaum zu vergleichenden Untersuchungen grösseren Umfanges anwenden lässt. Ob das Abnehmen der Tensionsstärke parallel mit der Resorption verläuft, oder ob die Gewebsreaktion vielleicht von anderen Faktoren abhängig ist, kann noch nicht als sicher entschieden betrachtet werden. Versuche in vitro zeigen, dass proteolytisches Ferment bei der Auflösung eine Rolle spielt; doch geben sie nur Auskunft über eine einzelne Seite des Resorptionsprozesses.

Zu vergleichenden Versuchen scheinen Tierversuche mit Beurteilung des histologischen Resorptionsbildes am besten geeignet. Die früheren Versuche lassen es wichtig erscheinen, dass der Faden in genau dem gleichen Gewebe und an der gleichen Stelle angelegt wird (vgl. *Goris & Rolland*). Die Gleichartigkeit des Tiermaterials ist hiernach eine selbstverständliche Forderung, ebenso wie das Ausseiden von Versuchstieren, bei denen die Resorption durch Gefässblutung oder Infektion gestört wird. Besonders wichtig erscheint die Wahl der Versuchstiere. *Kaninchen erscheinen absolut ungeeignet* auf Grund der sehr langsamen Resorption (*Lesser, Thompson u. a.*). Die Ursache hierfür ist vielleicht in einer besonders geringen Fermentproduktion zu suchen. In einigen orientierenden Versuchen haben wir in Übereinstimmung mit früheren Untersuchern Catgut noch 4 Monate nach Einnähen in der Schenkelmuskulatur von Kaninchen fast unbeeinflusst gefunden. Auch bei Meerschweinchen ist die Resorption sehr langsam (vgl. *Goris & Rolland*).

Eigene Versuche.

Wir haben erstens mit unseren Versuchen konstatieren wollen, dass unsere Technik, wenn sie bei gleichartigen Ratten als Versuchstieren in Serienversuchen von einer gewissen Grösse angewandt wurde, keinen Anlass zu individueller Variation gibt, eine selbstverständliche Voraussetzung für die Anwendbarkeit der Versuche überhaupt. Demnächst haben wir die Resorptionszeit für ein wärmesterilisiertes Catgut

(B)*) untersucht. Da auf der Grundlage von Tierversuchen keine übereinstimmenden Angaben darüber vorliegen, wie lang die Resorptionszeit von Catgut ist oder sein soll, haben wir vorläufig als Vergleichsgrundlage 2 Catgutpräparate (A und C)*) benutzt, die an chirurgischen Krankenhausabteilungen in Dänemark in grösserem Umfange verwendet werden. Die 3 Catgutpräparate wurden bei der Aussaat von grossen Mengen steril befunden. (Die Methoden werden in einer folgenden Arbeit veröffentlicht werden.)

Technik.

Als Versuchstier wurde die weisse Ratte verwendet, die ein Laboratoriumstier darstellt, das zu Serienversuchen geeignet ist und zugleich Catgut innerhalb verhältnismässig kurzer Zeit resorbiert. Die Ratten waren von der gleichen Zucht und wurden unter gleichartigen Laboratoriumsbedingungen ernährt. Es wurden nur männliche Ratten verwendet.

Suturierungsmethode. Der Faden wurde unter Äthernarkose in die vorderste Muskelgruppe des Femurs in Längsrichtung eingenäht. Bei vergleichenden Versuchen wurde das eine Catgutpräparat im linken, das andere im rechten Schenkel appliziert. Nach gründlicher Desinfektion der Haut wurde der Faden mit einer dünnen runden Nadel durchgezogen, indem das Bein am Fuss und an der Schwanzwurzel festgehalten wurde. Wenn man sich nach der Lage des Knochens richtet, ist es nach einiger Übung möglich, den Faden mit so grosser Genauigkeit zu legen, dass er die gleichen Muskeln und Faszien passiert, ohne grössere Gefässe und Nerven zu verletzen. Die Fadenenden werden direkt an der Haut abgeschnitten, und durch eine leichte Verschiebung der Haut verschwinden die Fadenenden nach innen, so dass die Öffnungen mit elasti-

*) A. Graf Catgut. (Jodgehalt ca. 4,8 %).

B. Novo Catgut. (Wärmesterilisiert nach vorausgehender Jodpräparation. Jodgehalt ca. 2,6 %).

C. Formalinbehandeltes, gekochtes Catgut. (Präpariert nach Rovsing's Methode).

schem Kollodium zugedeckt werden können. Wenn die Platzierung des Fadens richtig ist, sind die Bewegungen der Tiere nach 24 Stunden vollkommen natürlich.

Die Tiere einer Versuchsreihe wurden am gleichen Tage operiert und danach mit gleichmässigen Intervallen durch Gas getötet und die ganze Muskelgruppe zur Präparation entnommen.

Nach Fixierung in Zenker-Formol und Auswässern wurden die Präparate sorgfältig so zugeschnitten, dass die Schnitt-richtung möglichst rechtwinklig zur Längsrichtung des Fadens war.

Die histologischen Bilder entsprachen ganz der Beschreibung früherer Untersucher und können in den Hauptzügen in folgenden Stadien wiedergegeben werden:

- 1) Eine exsudative Entzündung überwiegend mit polymorphkernigen Zellen. Der Faden scheint unbeeinflusst. Der Grad der Reaktion wurde von *Bates* durch die Anzahl der den Faden umgebenden Zellschichten angegeben. In der vorliegenden Versuchsreihe haben wir die grössten und kleinsten Durchmesser von Faden und Reaktionszone mit dem Okularmikrometer gemessen und die Grösse durch die Anzahl der Teilstriche angegeben, wobei Objektiv Zeiss: 5, Okular: 2 verwendet wurde.
- 2) Ein proliferatives Stadium mit Auftreten von Fibroblasten, die nach und nach die Eiterzellen ersetzen. Die Oberfläche des Fadens zeigt beginnende Ausfransung.
- 3) Phagozytosestadium, in welchem das Granulationsgewebe zwischen die Fasern des Fadens eindringt und denselben in mehrere Teile spaltet.
- 4) Die endliche Resorption, wo der Faden von einem jungen Granulationsgewebe vollständig ersetzt ist.
- 5) Die festere Organisation und Schrumpfung dieses Bindegewebes zu einer strichförmigen oder runden Narbe.

Wenn die Tiere einer Versuchsreihe mit einem Intervall von 2 oder 3 Tagen untersucht werden, ist der Übergang zwischen den Stadien so gut charakterisiert, dass er zu Ver-

gleichen herangezogen werden kann. Ausser den Messungen haben wir benutzt:

- 1) Die beginnende Aufspaltung des Fadens (Fig. 1).
 - 2) Die Bildung einer gut abgegrenzten Bindegewebszone (Fig. 2).
 - 3) Das vollständige Verschwinden des Fadens (Fig. 3).
 - 4) Die Bildung einer fibrösen Narbe.
-

Versuch Nr. 1.

60 Ratten wurden mit Catgut B Nr. 0 in beiden Schenkeln genäht und in Gruppen von 10 Tieren mit 7 Tagen Zwischenraum untersucht, so dass wir für je einen bestimmten Zeitraum nach der Operation ca. 20 Präparate zum Vergleich hatten. Die Übereinstimmung zwischen diesen Präparaten war sehr gross, auch hinsichtlich der Messungen, wie aus der Tabelle 1 hervorgeht. Die während des Versuches abnehmende Grösse gibt den Aufschluss, dass der ganze Prozess unter einer gleichmässigen Schrumpfung in Übereinstimmung mit der Organisation des Bindegewebes verläuft. Die Messungen zeigten im übrigen nur wenig von Interesse und sind in den folgenden Versuchsreihen ausgelassen. Nur bei der Auscheidung der infizierten Fälle spielten die Masse eine Rolle. 3 Versuchstiere mussten wegen Infektion aus den Versuchen ausgeschieden werden. Die Abszesse waren in allen Fällen sehr charakteristisch durch die Grösse der Reaktionszone und die Persistenz von akuten Entzündungsphänomenen. In allen Fällen war die Resorption des Fadens weit weniger vorgeschritten als dem Zeitpunkt entsprach, was mit den Beobachtungen von *Fleming* (1876) übereinstimmt, die u. a. von *Fürle* bestätigt worden sind.

Aus dem Versuch geht im ganzen hervor, dass die Resorption in Rattenmuskulatur mit recht geringen Variationen verläuft, wenn Methode und Tiermaterial gleichartig sind.

Tabelle 1.

Versuchs- tag	An- zahl Präp. (Ver- suchs- tiere)	Catgutfaden			Reaktionszone		
		Aussehen	Kleinster Diam.	Grösster Diam.	Aussehen	Kleinster Diam.	Grösster Diam.
9.	22 (11)	Kompakt, mit leicht ausge- franzter Randzone	<u>15</u> (12—17)	17 (11—25)	Vorwiegend Leu- kozyten, eine An- zahl Fibroblasten	<u>43</u> (27—60)	60 (40—80)
16.	20 (10)	19 Fälle starke Aufspaltung, in 1 Fall mittelstarke. Masse: Grösster Fadenrest	<u>4</u> (2—5)	8 (3—24)	Vorwiegend Fibro- blasten, eine An- zahl Leukozyten	<u>33</u> (15—52)	63 (40—95)
23.	20 (10)	Faden nicht nach- weisbar			Gutabgegrenzte Zone von jungem Bindegew., keine Leukozyten	<u>15</u> (12—27)	34 (15—65)
30.	20 (10)				In 9 Fällen strei- fenförmige, in 2 Fällen runde Nar- be. In 9 Fällen fi- bröse Bindegewebszone	<u>8</u> (5—14)	19 (13—32)
37.	18 (9)				6 Fälle nichts nach- weisbar, 3 strei- fenförmige Narbe. In 8 Fällen fibröse Bindegewebszone. In 1 Fall Abszess	<u>5</u> (2—12)	9 (3—15)
44.	18 (9)				3 Fälle nichts nach- weisbar, 5 streifen- förmige Narbe. In 9 Fällen fibröse Bindegewebszone. In 1 Fall Abszess	<u>3</u> (2—5)	9 (4—17)



Fig. 1.

Beginnendes Einwachsen in den Faden (von unten her). Reaktionszone unscharf, bestehend aus einem Gemisch von Leukozyten und Fibroblasten.

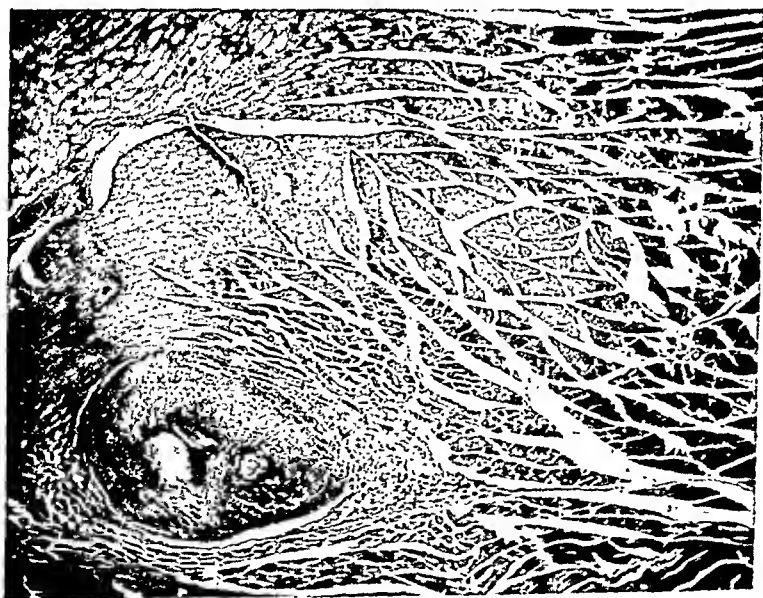


Fig. 2.

Faden aufgespalten, umgeben von einer gut abgegrenzten Zone von Bindegewebe.

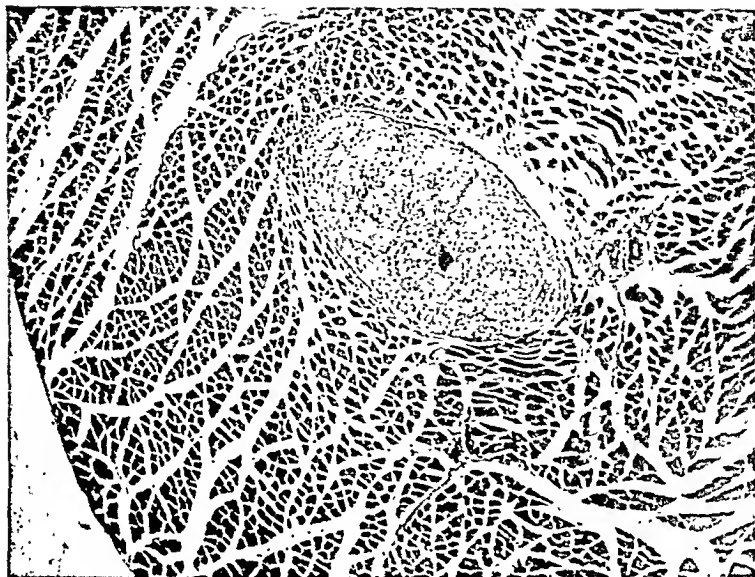


Fig. 3.

Faden verschwunden. Die Reaktionszone besteht aus einem gut abgegrenzten jungen Granulationsgewebe.

Versuch Nr. 2.

Ein Vergleich zwischen Catgut A und B wurde durch Einnähen in den linken bzw. rechten Schenkel in Serien von 17 Ratten entsprechend jeder Catgutstärke vorgenommen, im ganzen in 5 Serien. Die Tiere wurden mit Intervallen von 3 Tagen untersucht. Das Resultat ist in Fig. 4 wiedergegeben, wo links ein Vergleich des Verhaltens des Fadens (Aufspaltung und vollständiges Verschwinden), rechts der Gewebsreaktion vorgenommen ist. (Bildung einer gut abgegrenzten Bindegewebszone und einer festen Narbe).

Mit der Genauigkeit, die in dem einleitenden Versuch angedeutet ist, kann kein Unterschied in der Resorptionszeit von Catgut A und B beobachtet werden, indem die Aufspaltung des Fadens in beiden Fällen ca. am 15.—16. Tage stattfindet, und der Faden ca. am 21.—24. Tag endgültig resorbiert ist. Der etwas langsamere Verlauf der Gewebsreaktion

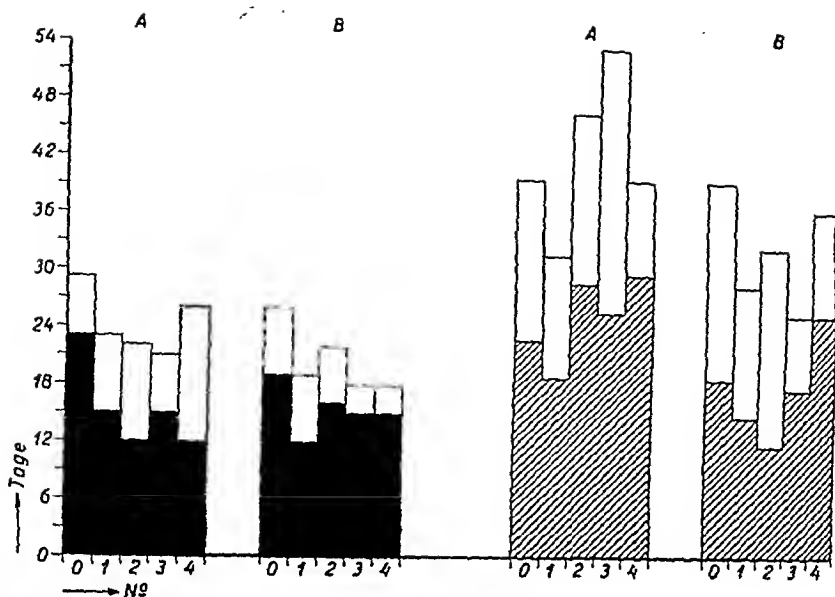


Fig. 4.

Vergleich zwischen der Resorption von Catgut A und B. Die beiden Figuren links zeigen die Zeitpunkte für die Aufspaltung (schwarze Felder) sowie für das vollständige Verschwinden des Fadens (weisse Felder).

Die beiden Figuren rechts zeigen die Zeitpunkte für die Bildung eines abgegrenzten Granulationsgewebes (schraffierte Felder) sowie für dessen Umbildung zu einer fibrösen Narbe (weisse Felder).

bei Catgut A ist vielleicht durch den etwas höheren Jodgehalt zu erklären.

Sehr auffallend ist die Resorption von Catgut Nr. 0, bei welchem sowohl die Aufspaltung wie die endgültige Resorption langsamer erfolgt als bei den dickeren Fäden. Diese Beobachtung, die den Versuchen von Wolff und Priestley an Menschen entspricht, deutet ebenso wie diese darauf hin, dass der dünne Catgutfaden oft das beste Suturmateriale ist, und dass in der Praxis durch die Verwendung dickerer Fäden nur eine grössere Initialstärke erreicht wird.

Ob die Ursache hierfür in einer Verschiedenheit der technischen Verarbeitung der Fäden zu suchen ist, worauf die verschiedene Resorptionszeit von Catgut Nr. 0 im Versuch

Nr. I und II hindeuten könnte, oder ob sie eine rein biologische Erklärung hat, ist von grundlegender Bedeutung für weitere Resorptionsversuche. Die Frage ist zur Zeit Gegenstand näherer Untersuchungen.

Versuch Nr. 3.

In diesem Versuch ist ein Vergleich zwischen Catgut B und C durch Einnähen an 17 Ratten vorgenommen. Nur Catgut Nr. 0 wurde untersucht. Fig. 5 zeigt, dass das durch Kochen sterilisierte Catgut eine bedeutend kürzere Resorptionszeit hatte als das jod- und wärmebehandelte, was in Übereinstimmung mit den Pepsinversuchen von *Kraissl & Meleney* ist.

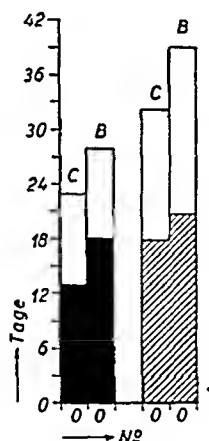


Fig. 5.

Vergleich zwischen der Resorption von Catgut C und B.

Die beiden Figuren links zeigen die Zeitpunkte für die Aufspaltung (schwarze Felder) sowie für das vollständige Verschwinden des Fadens (weisse Felder).

Die beiden Figuren rechts zeigen die Zeitpunkte für die Bildung eines abgegrenzten Granulationsgewebes (schraffierte Felder) sowie für dessen Umbildung zu einer fibrösen Narbe (weisse Felder).

Zusammenfassung.

1) Durch Serienversuche mit histologischer Untersuchung von 120 Muskelsuturen wird nachgewiesen, dass die Resorption von Catgut in Rattenmuskulatur mit sehr geringer Variation verläuft, wenn gleichartige Tiere und gleichartige Technik verwendet werden.

2) Durch Serienversuche wurde die Resorption der Fäden Nr. 0, 1, 2, 3 und 4 eines wärmeesterilisierten Catguts mit der eines chirurgisch durchgeprüften Präparates verglichen. In beiden Fällen begann die Aufspaltung des Fadens am 15.—16. Tag, und der Faden war ungefähr bei Ablauf der dritten Woche verschwunden. Die Gewebsreaktion scheint bei dem Catgutpräparat mit dem grössten Jodgehalt am längsten zu dauern.

3) Formalinbehandeltes, gekochtes Catgut hatte die kürzeste Resorptionszeit.

4) Die Resorption der verschiedenen Fadennummern verlief gleich schnell in Übereinstimmung mit früheren Erfahrungen der praktischen Chirurgie, und unterstützt somit die Auffassung, dass der einzige Vorteil bei der Anwendung dickerer Fäden in der Sicherung einer grösseren Initialstärke liegt. In einigen der Versuche zeigte Catgut Nr. 0 von zwei verschiedenen Fabrikaten eine bedeutend langsamere Resorption als die übrigen Nummern. Dieses Problem, das für weitere Resorptionsversuche von Bedeutung ist, bildet zur Zeit den Gegenstand näherer Untersuchung.

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VARIATIONS IN THE BACTERIAL FLORA OF THE INTESTINE OF WHITE RATS IN DIFFERENT CARBOHYDRATE DIETS.

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The history of the study of the bacterial flora of the intestine dates back to the first phase of the bacteriological era. When Metchnikoff in 1908 put forward his well-known hypothesis of the injurious effect of bacterial products of the intestine on the organism, these studies were greatly intensified. They were pursued by means of investigations of human material and also by experiments on animals. As a result of these studies considerable light has been shed on the action of the intestinal bacteria during the process of digestion. As a consequence of these results the flora can be divided into two separate, main groups: the carbohydrate-fermenting or so-called saccharolytic flora and the so-called proteolytic flora, which splits up the proteins during putrefaction. According to different diets the bacterial flora is dominated by either the one or the other of these two main groups. In individuals with an ordinary mixed diet a mixed flora is present. Among the bacterial occurring in the one or the other of these two groups the following are the most important (according to *Kendall and Haner, 1924*):

Saccharolytic bacterial flora:

Lactobacilli
Enterococci
Bacillus welchii

Proteolytic or putrefactive flora:	<i>Bacillus sporogenes</i>
	» <i>putrificus</i>
	» <i>mesentericus</i>
	» <i>histolyticus</i>
	» <i>proteus</i>
	» <i>pyocyaneus</i>

Like *B. welchii*, *B. coli* may occur in both these types of flora. Among the group of acidophilic lactobacilli are classed (according to *Topley and Wilson*) a group of pleomorphic Gram-positive bacteria, usually in the form of long, non-motile rods. They possess a property in common in being able to produce lactic acid by splitting lactic acid-producing carbohydrates and are capable of surviving the high degrees of acidity occurring ($\text{pH} = 3.0-4.5$). In this group we may distinguish *Lactobacillus bifidus*, mostly known as occurring in the faecal flora of breast-fed infants, Döderlein's bacillus, which *Cruickshank*, among others, considers to be identical with the third organism in the group, *Lactobacillus acidophilus* (Moro) or *Termobacterium intestinale* (Orla-Jensen), and finally *Lactobacillus bulgaricus*. The last-mentioned organism derives its name from Metchnikoff's discovery of its use as fermenting agent in the making of yogurt, which is drunk very much by the inhabitants of Bulgaria. *Lactobacillus acidophilus*, besides being present in the faecal flora of animals and man, occurs also in the oral flora and has been placed in a certain connexion with the causation of dental caries. The saccharolytic flora comprises mainly Gram-positive bacteria, which explains the frequently simplified terms for these types of floras, which are occasionally described as Gram-positive flora (saccharolytic) as opposed to Gram-negative flora (proteolytic). The intestinal bacteria of both man and animals are essentially alike, which has rendered the study of the variations of the bacterial flora possible by means of simple experimental technique. The white rat has been found to be the most suitable experimental animal and has therefore been used by the majority of investigators in

this field and was also employed in the present series of experiments.

As far back as 1886 *Hirschler* found that the typical putrefactive products in the faeces, indol and phenol, were considerably reduced following a diet of sugar, potatoes, glycerol and dextrin, whereas they were increased on a meat diet. *Winternitz* (1892) was the first to call attention to the capacity of milk to lessen putrefaction. He regarded this effect to be due to the lactose in the milk independent of the lactic acid produced. In experiments on dogs, among other test-subjects, *Herter* and *Kendall* (1908) studied the chemical components of the food and their relationship to the intestinal flora and found that a high carbohydrate diet tended to increase the fermentative flora and to depress the proteolytic. In the same manner *Jungano* (1909), by feeding white rats on a meat diet, obtained a predominance of a certain definite flora, afterwards defined as the proteolytic flora, with an excess of *B. coli*.

Since then numerous investigations have been made of the effect of carbohydrates on the bacterial flora of the intestine. The unvarying results of these investigations revealed that the acidophilic group of the saccharolytic flora could be readily increased by the addition of various carbohydrates to the diet and could occasionally be developed to a marked predominance. Thus *Sittler* and *Schiller* (1908) found that white rats fed on milk, lactose or dextrin showed an evident predominance of *B. bifidus*. This was confirmed in a similar investigation by *Distaso* (1914). By feeding white rats on raw liver and minced horse's meat *Gaspari* (1911) succeeded in obtaining an evident predominance of *B. coli* and *B. proteus*. *Rettger* and *Horton* (1914) also found that milk transformed the bacterial flora to a predominance of *L. acidophilus* and *L. bifidus*. In feeding experiments with corn starch they thought they observed an increase in the number of *L. acidophilus*.

Several thorough studies of the relationship of various carbohydrates to the lactobacillary group then followed. *Hull*

and *Rettiger* (1914) in careful studies on white rats found that milk and lactose were the only really effective substances that produced an »acidophilic« flora, i. e. with an evident predominance of this group of bacteria. Already after 2—4 days a change to an acidophilic flora could be effected. On the other hand, they failed to obtain any effect with dextrose, maltose, laevulose, galactose and dextrin. On the addition of saccharose they thought they were able to discern a slight increase of the acidophilic bacteria. *Sisson* (1917) succeeded in showing that the primary bacteria present in the milk did not play any role, since he obtained equally good results with pasteurized milk as with natural milk. In recent years *Herz* (1936) has shown that disiccated milk, too, has the same effect. On the other hand, no effect has been obtained with sour milk (*Henneberg* and *Henneberg*, 1935). Simultaneously with this increase of the acidophilic group there takes place a corresponding decrease of *B. coli*. This was shown by *Torrey* (1919) in a series of experiment on dogs, which when fed on lactose or dextrin also developed an acidophilic flora with an evident depression of *B. coli*. Glucose, saccharose and lactose as well as fat and starch failed to produce any effect. *Cruickshank* (1928) obtained essentially the same results: an acidophilic flora following an addition of lactose and dextrin, no effect when glucose, galactose, maltose or laevulose was added, whereas saccharose produced a very slight effect on the flora. In a very careful investigation of the same subject *Schieblich* (1929) was able to observe an abundant acidophilic flora alone following the addition of lactose, but only a slight development of the aciduric flora when dextrin was added. That a milk diet produces an abundant acidophilic flora in the intestine is also a well-known fact in human medical conditions. In infants there is a heavy excess of *Lactobacillus bifidus* and *L. acidophilus*, the former in natural breast-feeding, the latter in feeding with cow's milk.

Already in the early stages of the inquiry into this field the possibility of producing an acidophilic flora by peroral

administration of the organisms in question was discussed. *Rettger* and *Kendall* (1911) succeeded in doing so by supplying an emulsion in saline of *L. acidophilus* per os, and they were able to show a change of the intestinal flora of rats and of man to the aciduric type. On the other hand, experiments with *B. bulgaricus* were not so successful, the organism being unable to establish itself in the intestine (*Rettger* and *Cheplin*, 1921). *Cruickshank*, too, was able to obtain a certain effect with this emulsion of *L. acidophilus* when fed by the mouth, but a considerably greater increase of the predominance of the acidophilic flora was obtained when lactose was added at the same time. These observations have been confirmed by other workers, for instance, *Sanborn* (1931), *Mejblo* and *Nygart* (1939), who obtained their results in tests on human material, and others who employed so-called »acidophilus-milk« for therapeutic use.

Interesting observation made in recent years, however, have shown that also other factors besides milk or lactose alone may influence the acidophilic flora of the intestine. In feeding experiments with a salt-deficient diet *Eppricht*, *Valley* and *Smith* (1937) obtained a marked depression of the acidophilic group with a corresponding predominance of *B. coli*. The addition of sodium, potassium, calcium or phosphorus alone failed to restore the flora to the acidophilic type, a return to any considerable extent to this acidophilic group being effected only after the administration of a particular optimal saline solution (Osborne-Mendel's salt mixture). That certain species of fruits are also capable of stimulating the acidophilic group has been demonstrated by *Weinstein* and *Weiss* (1937), who in feeding experiments with banana, apple, raisin, strawberry and whortleberry obtained an increase of this group of bacteria, but failed in experiments with dried tomatoes and plums.

At the same time as the transformation of the bacterial flora of the intestine to an acidophilic type there is also a marked lowering of the pH value. This is an invariable observation made by those investigators who have been able

to transform the flora to this direction by means of lactose and milk. Thus *Cannon* and *McNease* (1923) found a pH of 4.4 in the caecum of rats fed on lactose while that of the controls was 6.6 and the animals which developed a proteolytic flora on an exclusive meat diet showed a pH of 7.0. Thus the same diet as developed an acidophilic flora showed also on the whole a low pH value. That there is a connexion between an acidophilic flora and a low pH value has also been proved by *Weinstein*, *Weiss* and *Gillispie* (1938). They fed different groups of animals on meat alone and on meat and lactose and obtained in the latter, on the average, a higher number of *B. acidophilus* and a lower pH value. They were unable, however, to ascertain any absolute correlation.

There has been much discussion as to whether this acidity is due to the activity of the acidophilic bacteria or is produced by the milk or the lactose during fermentation. Contrary to *Rettger* and *Cheplin*, who are of opinion that the increased degree of acidity in the intestine is due entirely to the effect of *Lactobacillus acidophilus*, *Cannon* and *McNease*, *Beach*, *Kessel*, *Weinstein*, *Weiss* and *Gillispie*, and others maintain that the most important factor is lactose, which produces this acidity by the formation of lactic acid. Milk and the lactose present in it are partly broken down by fermentation in the intestine during the formation of lactic acid, which is a more potent acid than, for instance, acetic acid (the dissociation constant of lactic acid is 1.4×10^{-4}). Thus the addition of lactose provides the acidophilic bacteria with an optimal medium and on the fermentation of lactic acid they acquire an optimal acidity value. This undoubtedly provides the valid explanation why this group of acidophilic bacteria increases so rapidly in numbers and dominates other groups of bacteria. That both factors, medium and bacteria, cooperate and that both lead to this degree of acidity has also been emphasized by *Cruickshank*, among others. According to careful experiments made by *Rosebery* (1932), the lactobacilli in growing produce extremely high degrees of acidity. This depends of course partly on the degree of bacillary growth,

the pH falling parallel with the growth of the culture, partly on the nutrient medium. Under optimal conditions the pH is lowered within 24 hours from 7.0 to 3.2 — 4.0 i. e. to such values that even *B. coli*, whose acid tolerance is $\text{pH } 0 \approx 3.58$, is strongly inhibited and may even be suppressed altogether. In view of these facts the question may be asked whether both a suitable medium and lactic acid-producing bacteria with a high formation of acid and a strong acid-tolerance do not cooperate in the production of acid, as suggested by *Cruickshank*.

The importance of the bacterial flora of the intestine in diseases has aroused great interest. *Metchnikoff's* theory implies that the process of putrefaction going on in the intestine continually produces products poisonous to the organism, which in time leads to atrophy and a shortened period of life. All agents capable of limiting this putrefactive process thus tend to prolong life. *Metchnikoff* himself considered sour milk, particularly yogurt, to be such an agent. It also became very soon evident that lactic acid and lactic acid-producing bacteria had a depressive effect on putrefaction. In order to investigate the influence of such a putrefaction on various vital processes *Schieblich* (1929) undertook a series of experiments on rats for the purpose of discovering the effect of diet on growth, propagation, blood picture and resistance to disease, but was unable to find any difference in these respects between animals fed on a diet that developed a putrefactive flora in the intestine and those fed with milk or lactose. Thus no scientific evidence has been advanced so far in support of *Metchnikoff's* theory, and it can be safely said that it is devoid of present-day interest.

Acidophilus milk has been recommended by many as a therapeutic aid in enterocolitis for the purpose of transforming a putrefactive flora to an acidophilic. A similar effect may also be obtained with milk. Opinions differ however as to the effect of such a therapy; some investigators still maintain that this bacteriotherapy is beneficial, others are of opinion that it has not fulfilled expectations.

Previous investigations have thus shown by what means the intestinal flora can be changed to a marked predominance of either of the two groups, the saccharolytic, the main component of which is the sensitive acidophilus group, and the proteolytic. For the latter of these types an exclusive meat diet without an admixture of large quantities of carbohydrates is required. For the former lactose or milk is necessary. Opinions differ as to whether any real effect can be obtained with dextrin. *Eppricht, Valley and Smith*, are of opinion that no effect can be obtained with dextrin. As mentioned above, a marked effect can also be obtained with certain fruits.

Experimental.

The experiments described below were performed in conjunction with the studies of the importance of saccharose from a nutritive physiological point of view carried on at the Institute of Medical Chemistry of Lund. In the present work attention has been paid to the bacteriological aspect of the problem, a comparison having been made between the action of starch and that of saccharose on the bacterial flora of the intestine. Neither of these carbohydrates is manifestly capable of effecting a transformation of the bacterial flora to a dominating acidophilic type. Within the group of carbohydrates these two substances occupy different positions, one of them being a disaccharide, the other a (colloidal) polysaccharide. Saccharose breaks up early in the intestinal canal into the monosaccharides glucose and fructose, while starch is probably first converted into dextrins and maltose and to a certain extent glucose, whereas the conversion of maltose into glucose and of dextrin into lower decomposition products with glucose, too, as one of the final products probably takes place during almost the entire passage along the intestinal canal. The fermentation of starch probably also requires a longer time. It may also be assumed that following a starch diet the carbohydrates are present to a greater extent also in the lower intestinal regions than on a saccharose diet alone.

Thus these two substances differ from each other both chemically and with respect to their absorption in the intestine.

The experiments were performed on white rats, which received a fully adequate diet as far as nutrition and vitamins are concerned, but one group was given carbohydrates in the form of cane sugar alone, and the other group only starch. The animals were kept on this diet during the entire experimental period, no change from a normal mixed diet to the experimental diet being made. The basal diet of the animals was composed as follows:

The cane sugar animals received the following diet:

Saccharose	69	per cent.
Casein	20	» »
Peanut oil	2	» »
Salt mixture	4	» »
Yeast	5	» »

To this diet was added 5 grams of cod-liver oil per 1 kilo body-weight. The food mixture was mixed with water to a consistency suitable for the animals. The salt mixture used was MacCullum's No. 185. The starch animals were given a similar food mixture but with a substitution of an equivalent quantity of rice starch for the saccharose.

The faecal flora of the two groups of experimental animals.

The faecal flora was studied in direct smears stained by Gram's method. Further, as described below, it was possible to study the number of *B. acidophilus* by means of cultivation on a special medium. The acidophilic group, which was at once found to be the only group where a real difference was discernible, possesses easily recognisable morphological properties already in the direct smears, and as far as the study of this group is concerned this rather simple method may be conveniently used. *Retzger* and *Cheplin* has compared this method with methods of cultivation and found it to be quite

satisfactory, the only drawback being that one has no guarantee that all the bacteria thus observed are alive. In such an investigation as the present, where a comparison is made between two fully comparable groups, this will not constitute a source of error. This method was also employed by *Cruickshank*. In the examination described below the technique employed was as follows: A weighed quantity of faeces was diluted with physiological saline in a dilution of 1/30, which was then emulsified by stirring to a satisfactory degree of homogeneity. A drop of this emulsion was smeared on a slide, stained by Gram's method and the numbers of bacteria of the different bacterial groups per visual field counted. Three visual fields were thus counted and the mean of these counts was obtained. In the variegated picture presented by the bacterial flora of the intestine of white rats, which has been carefully studied by *Schieblieh*, *Rettger* and *Cheplin* and by *Frost* and *Huntington*, and others, it was found already at the beginning of the examination unnecessary to differentiate each special group, partly because, with the exception of the acidophilic group, they did not show any marked variation, partly because the morphological property in a direct smear would be an unsatisfactory basis for a careful study of the quantity of different groups except this acidophilic group. For the purpose of this investigation, the objective of which was to note the difference between the two groups with respect of the bacteria that really showed marked variation, it was found sufficient to divide morphologically the bacterial flora present in direct smears into 5 large groups:

- | | |
|----------------|-------------------------------|
| Gram-positive: | Enterococci |
| | Lactobacilli |
| | Other Gram positive organisms |
| Gram-negative: | The Coli-proteus group |
| | Other Gram-negative bacteria. |

The lactobacillus acidophilus and closely allied lactobacilli are morphologically characteristic and easily distinguishable. They appear here in the form of Gram-positive rods of varying

length, sometimes in the form of chains, and occasionally in the shape of a Y. Further, their identity as lactobacilli could be established after cultivation on an acetic acid-containing agar medium, when they showed the fermentative properties of these bacilli. No distinction was made in these counts between *L. acidophilus* and *L. bifidus*, as that was considered to be rather outside the scope of the present investigation. They agree in all probability with the micro-organisms classified by *Orla Jensen* and *Winther* as *Thermobacteria*.

Tables 1 and 2 show the values obtained in counts of the bacterial groups in direct smears from the faeces of these rats. Even on a superficial examination it was evident that in spite of individual variations the numbers of acidophilic lactobacilli were much greater in the faeces of the animals fed with starch than in that of the animals fed with cane sugar. As seen from Table 3, the mean value of the percentage also shows an evident difference, which is statistically significant. The difference between the means of acidophilic bacteria in the two groups is evidently greater than

$$2\sqrt{\Sigma_1^2 + \Sigma_2^2}$$

in this case: $2\sqrt{1.2^2 + 0.15^2} = \pm 2.4$ Diff. = 9.5

While the percentage of acidophilic lactobacilli in the group of »cane sugar animals« does not amount to 1 per cent. of the total number of bacteria, in the group of »starch animals« the percentage of acidophilic bacilli is more than 10 per cent. Other groups of bacteria show individual variations but the mean values of the two groups are approximately equal. The differences that seem to occur, however, are so small that they cannot be determined by this statistical computation. The difference between the means of the *Coli-Proteus* groups is greater than

$$2\sqrt{\Sigma_1^2 + \Sigma_2^2} ,$$

in this case $2\sqrt{1.71^2 + 1.96^2} = 6.68$ Diff. = 5.3. Thus the difference is not statistically significance. Probably some depression of the Gram-negative group takes place, but it is

Table 1.

No. of animal	Mean value	Animals fed with saccharose					
		Gram-positive bacteria			Gram-negative bacteria		
		Enterococci	Acidophilus	Other organisms	Coli- Proteus	Other organisms	Total number of bact.
3330	Mean value	84	5	12	245	85	431
	%	19.5	1.2	2.8	50.8	19.7	
3321	Mean value	412	1	15	383	350	1101
	%	35.5	0.1	1.3	33.0	30.2	
3275	Mean value	171	4	35	350	261	826
	%	20.7	0.5	4.8	42.4	31.6	
3274	Mean value	104	7	43	110	85	349
	%	29.8	2.0	12.3	31.5	24.4	
3264	Mean value	97	8	28	121	179	433
	%	22.4	1.8	6.5	27.9	41.3	
3259	Mean value	139	8	11	132	53	343
	%	40.5	2.3	3.2	38.5	15.5	
3352	Mean value	413	25	36	611	158	1273
	%	32.4	2.0	2.8	50.4	12.4	
3347	Mean value	121	5	14	1171	216	1527
	%	7.9	0.3	0.9	76.7	14.2	
3380	Mean value	170	5	1	483	152	811
	%	21.0	0.6	0.1	59.6	18.7	
3378	Mean value	199	10	40	347	87	683
	%	29.1	1.5	5.8	50.8	12.7	
3371	Mean value	81	5	6	483	392	967
	%	8.4	0.5	0.6	49.9	40.5	
3725	Mean value	183	5	10	576	10	783
	%	23.4	0.6	1.3	73.6	1.1	
3777	Mean value	262	7	27	330	173	799
	%	32.8	0.9	3.7	41.3	21.7	
3825	Mean value	135	7	2	436	366	946
	%	14.3	0.7	0.2	46.1	38.7	
3811	Mean value	143	3	17	820	773	1756
	%	8.1	0.2	1.0	46.7	44.0	
3870	Mean value	76	4	7	505	318	910
	%	8.4	0.4	0.7	55.5	34.9	
3971	Mean value	61	6	44	399	244	754
	%	8.1	0.8	5.8	52.9	32.4	
3835	Mean value	145	1	2	601	515	1264
	%	11.5	0.1	0.2	47.5	40.8	
3865	Mean value	175	18	8	1076	514	1791
	%	9.8	1.0	0.5	60.1	28.7	
3841	Mean value	62	9	77	1069	909	2128
	%	2.9	0.4	3.6	50.2	42.7	
Mean value %		19.3	0.9	2.9	49.5	27.3	997
		± 2.43	0.15	0.66	2.71	2.70	109

Table 2.

No. of animal	Mean value	Animals fed with starch					
		Gram-positive bacteria			Gram-negative bacteria		
		Enterococci	Acidophilus	Other organisms	Coli-Protus	Other organisms	Total number of bact.
3289	Mean value	37	9	5	93	78	222
	%	16.7	4.1	2.3	41.9	35.2	
3515	Mean value	232	236	129	672	173	1442
	%	16.1	16.4	8.9	46.6	12.0	
3523	Mean value	179	141	92	184	137	733
	%	24.4	19.2	12.5	25.1	18.7	
3521	Mean value	173	92	33	227	137	662
	%	26.1	13.9	5.0	34.3	20.7	
3585	Mean value	89	38	30	289	135	581
	%	15.3	6.5	4.1	49.7	23.2	
3293	Mean value	18	36	19	172	214	459
	%	3.9	7.8	4.1	37.5	46.6	
3226	Mean value	52	51	10	93	94	300
	%	17.3	17.0	3.3	31.0	31.3	
3227	Mean value	53	27	10	153	49	288
	%	18.4	9.4	2.1	53.1	17.0	
3375	Mean value	59	29	23	143	91	345
	%	17.1	8.4	6.7	41.4	26.4	
3693	Mean value	82	84	5	170	64	405
	%	20.2	20.7	1.2	42.0	15.8	
3671	Mean value	44	35	8	118	66	271
	%	16.2	12.9	3.0	43.5	24.4	
3861	Mean value	52	36	16	190	100	394
	%	13.2	9.1	4.1	48.2	25.3	
3735	Mean value	117	61	7	311	126	622
	%	18.8	9.8	1.1	50.0	20.3	
3847	Mean value	159	145	3	1650	620	2577
	%	6.2	5.6	0.1	64.0	24.1	
4021	Mean value	142	85	32	462	320	1041
	%	13.6	8.2	3.1	44.4	30.8	
3895	Mean value	291	97	11	638	365	1402
	%	20.8	6.9	0.7	45.5	26.0	
3900	Mean value	91	35	10	549	508	1193
	%	7.6	2.9	1.1	46.0	42.5	
3683	Mean value	69	71	10	616	724	1492
	%	4.6	4.8	0.8	41.3	48.5	
4023	Mean value	68	174	25	669	285	1221
	%	5.6	14.3	2.1	54.8	23.3	
Mean value %		14.8	10.4	3.5	44.2	26.9	
		± 1.46	1.19	0.69	1.96	2.26	

Table 3.

Comparison between the percentages of different bacterial groups in the intestine of animals fed with saccharose and animals fed with starch.

	Gram-positive			Gram-negative	
	Entero cocci	Acido-philus	Other bact.	Coli-Proteus	Other bact.
Percentage of bacterial groups in animals fed with <i>saccharose</i> :	19.3	0.9 ± 0.15	2.9	49.5	27.3
Percentage of bacterial groups in animals fed with <i>starch</i> :	14.4	10.4 ± 1.2	3.4	44.2	26.9

not evident in the relatively slight, but quite establishable, difference that *L. acidophilus* shows between the two groups.

From the results thus obtained by a direct examination of the smears from the faeces it also appeared probable that culture experiments, too, would show some variation between the acidophilic groups in the two groups of animals. For that reason the following experiment was carried out. An emulsion with the exact dilution of 1/300 was prepared from a weighed quantity of the faeces of each animal. The medium used was a strongly acid agar, which permits the development only of *lactobacillus acidophilus*. An acetic acid medium, recommended for such a purpose was therefore employed. To 2 per cent. glucose-agar was added up to 0.12 per cent. acetic acid. This percentage was obtained by titration of the amount of acid permissible for the development of *L. acidophilus* on a 2 per cent. glucose-agar. From a Pasteur pipette, which was of exactly the same calibre for one animal from each group, a drop of the faecal emulsion was placed on a slide. With the aid of a platinum loop this drop was then uniformly spread over the surface. The colonies, which appear as small dewdrops after 24 hours, can be easily counted. The result of this experiment is shown in Table 4.

From Table 4 it will also be noted that there is an evident

difference between the number of *L. acidophilus* present in the faecal flora of the two groups of animals. The number of colonies from the »starch animals« was about 10 times greater than that from the »cane sugar animals«.

Table 4.

Cultures of *Lactobacillus acidophilus* grown on a glucose acetic acid medium from a definite quantity of a faecal emulsion from animals fed with cane sugar and animals fed with starch.

Cane sugar animals		Starch animals	
No. of animal	No. of colonies	No. of animal	No. of colonies
3870	40	3899	120
3880	32	3904	350
3884	46	3860	200
3877	30	3892	300
3865	60	3895	250
3942	85	3906	800
Total:	293	Total:	2220
Mean value:	45.5	Mean value:	370

Variations in the degree of acidity of the intestinal contents in both groups of animals.

Since the degree of acidity has always been associated with the number of lactobacilli present in the intestinal flora, this relationship was also examined in the animals fed on saccharose, which showed only small numbers of these organisms, and those fed on starch, in which the organisms were more abundant. The data given below concerning the pH of the alimentary canal following a starch or saccharose diet respectively have been supplied by *Lanke*, and are recorded in an unpublished investigation of this problem at present being carried on at the Institut of Medical Chemistry of Lund.

The determinations of the pH of the contents of the intestine were made in the following manner: Immediately after the animal had been killed by decapitation, the intestinal canal was removed and the contents of the different sections

plated under liquid paraffin (to prevent the substitution of gas by air). The pH of the intestinal contents was then measured with the aid of a glass electrode, two buffers being prepared for each sample, one with a known pH value below and the other with a pH value above the sample in question. The known pH values of these buffers were set at intervals of $\frac{1}{2}$ units.

The result of these estimations of the pH value of the contents of the large intestine is summarized in Table 5. From this table it will be seen that the pH of the contents of the lower colon in the animals fed on starch was $(7.7 \pm 1.2) = 10^{-7}$ as compared with a pH of $(1.8 \pm 0.2) = 10^{-7}$ in the animals fed on sugar. In these experiments this variation must be put in connexion with both the food and the flora present in the large intestine. In estimations in exactly similar animals *Lanke* also finds differences in other parts of the digestive canal, e. g. in the stomach, but these differences must be due to other conditions. On comparison with the values obtained in a highly predominant acidophilus flora there seems to be an agreement in so far that a higher percentage of lactobacilli is accompanied by a higher degree of acidity.

Table 5 a.

Starch animals		
	caecum	lower colon
3987 ♀	—	6.27
4054 ♀	6.45	6.01
4063 ♀	6.41	6.32
3990 ♀	6.63	6.18
4057 ♀	6.30	6.28
4087 ♀	6.48	6.48
3981 ♂	—	6.35
4051 ♂	6.51	6.45
4050 ♂	5.90	5.83
3989 ♂	6.17	6.12
4055 ♂	6.25	5.85
4081 ♂	6.91	—
Average (estimated from the		
hyd. ion conc.)	6.32	6.11

Table 5 b.

Sugar animals		
	caecum	lower colon
4037 ♀	6.89	6.76
4041 ♀	6.70	—*
4042 ♀	6.64	6.62
3976 ♀	7.04	6.95
4033 ♀	6.96	6.62
3979 ♀	—	6.61
4035 ♂	7.09	7.09
4038 ♂	6.97	—*
4036 ♂	7.29	7.08
3975 ♂	7.06	6.85
4039 ♂	6.97	6.91
3921 ♂	—	6.49
Average (estimated from the hyd. ion conc.)		
	6.92	6.75

*) No intestinal contents.

Discussion.

Earlier examinations of the bacterial flora following different carbohydrate diets have shown that feeding with milk and lactose rapidly caused a great increase in the number of lactobacilli. The increase observed on this diet reaches values of 60—90 per cent. of the bacterial flora (*Cruickshank*). This absolute dominance is mostly due to the fermentation of these substances with formation of lactic acid, which the lactobacilli are able to withstand to a much greater extent than other intestinal bacteria. Another important factor in this dominance of the lactobacilli in these lower regions of the intestinal canal is undoubtedly the fact, as also pointed out by *Distaso* (1914), that the lactose is resorbed in the lower regions of the intestinal and extending for a long distance may form a medium for the growth of the lactobacilli. Further, lactose is known to possess the lowest assimilation limit of all sugars, a fact, as also pointed out by

Rettger and *Cheplin*, that should always be borne in mind in the discussion as to the cause of the dominant acidophilic flora in a lactose diet. The degree of acidity caused by the formation of lactic acid has been attributed by the majority of investigators to the diet. Since the lactobacilli are one of the main factors in maintaining this fermentation of lactic acid they may of course be regarded as a not unimportant factor in keeping the faeces acid. As emphasized by *Cruickshank* and *Herz*, these two factors must of course be taken into consideration in the discussion as to the cause of the low pH occurring.

Our task in the examinations described above was to study the bacterial flora under conditions in which lactose or milk or such fruits as may have an influence on acidophilus are present in the diet. The carbohydrates given to the experimental animals are those usually present in our food and having varying rates of fermentation and absorption, saccharose and starch. In the present feeding experiments it was also proved that the lactobacilli were the only sensitive group of bacteria. In feeding with saccharose, which is rapidly resorbed in the higher regions of the intestine, *L. acidophilus*, as expected, was very scanty, not more than 0.9 per cent. of the entire flora. If starch is used the resorption is moved to the lower regions of the intestine, and the colon flora obtains a greater admixture of carbohydrate. In this case the lactobacilli show a marked reaction and increase to 10 per cent. This variation of course cannot be called acidophilic, and that should be the reason why this fact has not previously been taken into consideration. At the same time the pH value falls from 6.80 to 6.16. *It is therefore evident that the lactobacilli increase in numbers with an increase in the amount of carbohydrate in the intestine, even if no lactic acid-producing sugars are present.* This may be taken as an illustration of the sensitivity of the bacterial flora of the intestine in various carbohydrate diets. In fermentative dyspepsia, when an abnormal fermentation takes place in enterocolitis, carbohydrates that are resorbed only in the upper

regions of the intestine are recommended for use in the diet (Svartz). That this is an important factor in the development of the lactobacilli of the saccharolytic flora is obvious.

The degree of acidity, too, increases along with the number of the lactobacilli in the lower region of the intestine even if no fermentation of lactic acid takes place. This increased acidity may be due either to the action of the lactobacilli themselves or to a carbohydrate fermentation with formation of acids, or it may be due to both these factors. The most important constituent is probably the production of acids during the fermentation of carbohydrates. The degree of acidity and the number of lactobacilli do not increase on a starch diet to such an extent that a statistically significant effect on the coli group could be ascertained.

Thus it is evident that a starch diet alone, as compared with a saccharose diet alone, has a marked influence on the lactobacillary group of the bacterial flora. The variation occurring is evidently suited to the different chemical relationships in the intestinal canal during the different conditions.

Summary.

The bacterial flora of the intestine was examined in two groups of white rats given an adequate diet as regards calories and vitamins. One group received only carbohydrates in the form of saccharose and the other group in the form of starch.

1. By means of counts of different groups of bacteria in direct smears it was ascertained that lactobacilli amounted to 10 per cent. of the entire bacterial flora following a starch diet, whereas these organisms were scanty on a cane sugar diet, only 0.9 per cent.

2. In cultivation on a particularly suitable medium to these lactobacilli about 10 times more colonies were obtained from the same measured amount of faecal emulsion from starch-fed animals than from that of animals fed on cane sugar.

3. Simultaneously with an increase in the number of acidophilic lactobacilli a lower pH value of the faeces was obtained.

4. The group of acidophilic lactobacilli obtains a favourable environment through such carbohydrates as are resorbed late and are present in the lower intestine.

5. The lactobacilli are favoured by the presence of carbohydrates in the lower part of the intestinal canal, even if lactose is not contained in the diet. An increase in the number of acidophilic bacilli to such an extent that the flora can be called wholly acidophilic, as in a milk or lactose diet, cannot be obtained with the carbohydrates occurring in an ordinary diet.

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ÜBER DAS VORKOMMEN VON SALMONELLA- ANTIGENEN IN COLI-KULTUREN.

Von F. Kauffmann.

(Eingegangen bei der Redaktion am 24. Juli 1940).

Nachdem ich im Laufe der letzten Jahre von ausserhalb verschiedene Coli-Kulturen erhielt, die Salmonella-Antigene besitzen, will ich jetzt hierüber im Zusammenhange berichten, zumal es sich hierbei nicht nur um ein spezielles Salmonella- und Coli-Problem, sondern gleichzeitig auch um das Problem der »Paragglutination« im allgemeinen handelt.

Bei der von Kuhn und Woithe zuerst beschriebenen »Paragglutination« handelt es sich nach Kuhn, Gildemeister und Woithe um einen spezifischen Immunitätsvorgang und zwar dadurch bedingt, dass von Hause aus saprophytische Keime im pathogenen Milieu des Darmes allmählich Receptoren für die Agglutinine des Dysenterieserums gewinnen. W. Silberstein, der sich in seiner Arbeit »Zur Frage der Spezifität der Ruhrparagglutination« mit dieser Auffassung und den hiergegen von Breinl erhobenen Einwänden auseinandersetzte, gelangte auf Grund seiner experimentellen Untersuchungen zu dem Schluss, dass die ursprüngliche Deutung der Ruhrparagglutination als einer spezifischen Immunitätsreaktion entgegen der Auffassung von Breinl zu Recht bestehe. (Betr. weiterer Literaturangaben sei auf die Arbeit von W. Silberstein verwiesen).

Obwohl bereits in verschiedenen älteren Arbeiten über Paragglutination das Vorkommen von Coli-Kulturen, die z. B. von Typhusseren agglutiniert wurden, erwähnt wird, so ist

*) Diese Arbeit wurde mit finanzieller Unterstützung des Commonwealth Fund ausgeführt.

auf Grund der damaligen Antigenkenntnisse in keinem Falle der Beweis geführt worden, dass es sich hierbei um bestimmte, genau definierte *Salmonella* O- und H-Antigene gehandelt hat. Erst in einer Arbeit von *H. Habs* und *E. Arjona* aus dem Jahre 1935 »Ueber einen Stamm von *Bacterium coli* mit Antigenbeziehungen zur *Salmonellagruppe*« wird auf Grund näherer Antigenanalysen ein Colistamm beschrieben, der ein Partial-O-Antigen und zwar einen Teil des übergreifenden XII-Antigens besitzt.

Im Jahre 1937 berichtete dann *S. Gard* über einen Colistamm mit *Salmonella* H-Antigen und setzte diese Untersuchungen im Jahre 1939 zusammen mit *E. J. Eriksson* in der Arbeit »Studien über Coli-Stämme mit *Salmonella*-H-Antigenen« fort.

Im Folgenden will ich nun über verschiedene Stämme der Coli-Gruppe mit *Salmonella*-Antigenen berichten und die Bedeutung dieser Befunde erörtern.

Das untersuchte Material.

I. Coli-Kulturen mit Salmonella Vi- und H-Antigenen.

- 1) Coli 2624/36 von *A. Gnosspelius*, Göteborg, erhalten, mit der Angabe, dass diese Kultur unspezifisches H-Antigen der *Salmonella*-Gruppe enthalte.
- 2) Coli 6179/36, 1743/37 und 5396/38 von *S. Gard*, Stockholm, erhalten, mit der Angabe, dass diese 3 Kulturen unspezifisches H-Antigen der *Salmonella*-Gruppe enthalten.

Das Vorliegen des Vi-Antigens in diesen 4 Kulturen wurde weder von *Gnosspelius* noch von *Gard* festgestellt.

II. Coli-Kulturen mit Salmonella H-Antigenen.

- 1) Coli 491/36 von *A. Gnosspelius*, Göteborg, erhalten, mit der Angabe, dass diese Kultur unspezifisches H-Antigen der *Salmonella*-Gruppe enthalte.
- 2) Coli 5821/38 von *S. Gard*, Stockholm, erhalten, mit der Angabe, dass diese Kultur unspezifisches H-Antigen der *Salmonella*-Gruppe enthalte.

III. Coli-Kulturen mit *Salmonella* O-Antigenen.

- 1) Coli »Zürich« von H. Schütze, London, erhalten, mit der Angabe, dass diese Kultur fast das ganze O-Antigen von *S. paratyphi* B enthalte.
- 2) Coli »Ka.« von N. Černozubov, Zagreb, erhalten, mit der Angabe, dass diese Kultur die I. IV-Antigene enthalte.
- 3) Coli »Zicht 11391« von F. Schiff, New York, erhalten, mit der Angabe, dass diese Kultur das gesamte O-Antigen von *S. onderstepoort* (I). VI. XIV. XXV. enthalte.

I. Über Coli-Kulturen mit *Salmonella* Vi- und H-Antigenen.

Bei den 4 hierher gehörenden Coli-Kulturen mit Vi- und H-Antigenen handelt es sich um *einen* bestimmten Typus, da diese 4 Stämme in kultureller und serologischer Hinsicht völlig übereinstimmen. Auf das kulturelle Verhalten dieser Stämme will ich weiter unten im Zusammenhange eingehen. In Bestätigung der Angaben von A. Gnosspeilius, die mir persönlich gemacht wurden, und der Literaturangaben von Gard resp. von Gard und Eriksson enthalten diese 4 Coli-Stämme gut entwickeltes *Salmonella* H-Antigen der unspezifischen Phase und stehen der 1,5... Phase des *Kauffmann-White-Schema* nahe. Es sei in dieser Hinsicht auf die Angaben von Gard und Eriksson verwiesen.

Bei der Untersuchung des Stammes 2624/36 fiel mir bei der Betrachtung einzelner Kolonien auf Agarplatten im durchfallenden Lichte das zuerst von A. Giovanardi beschriebene Dissoziationsphänomen auf, das darin besteht, dass man bei Typhuskulturen den von mir beschriebenen V-W-Formenwechsel makroskopisch an dem trüben resp. klaren Aussehen der einzelnen Kolonien erkennen kann. In einer vorhergehenden Arbeit (J. of Hyg. 1940) über einen neuen *Salmonella*-Typus mit Vi-Antigen (*S. ballerup*) haben F. Kauffmann und E. Moller dasselbe Phänomen beschrieben, jedoch leider vergessen, die soeben erwähnte Arbeit von A. Giovanardi zu zitieren. Die Befunde von A. Giovanardi konnten also nicht nur bei *S. typhi* und *S. ballerup* (XXIX. Vi. z_{14}), sondern jetzt auch an Coli-Kulturen bestätigt werden.

Betrachtet man eine 20std. Agarplatte, auf der sich zahlreiche einzelne Kolonien des Coli-Stammes 2624/36 befinden, im durchfallenden Lichte, so kann man sehr deutlich zwischen trüben und klaren Kolonien unterscheiden und zwar in genau derselben Weise wie bei *S. typhi* oder *S. ballerup*. Die trüben Kolonien (V-Form) ergeben bei der Objektglasagglutination in einem Typhus Vi-Serum eine prompte und starke Vi-Agglutination, während die klaren Kolonien (W-Form) negativ reagieren. Bei Weiterzüchtung der trüben Kolonien (V-Form) kam es immer wieder zur Abspaltung der klaren Kolonien (W-Form), während umgekehrt bei Fortzüchtung der W-Form nur selten und oft erst nach mehreren Passagen V-Formen vereinzelt auftraten.

Bei der Fortzüchtung der V-Form ereignete es sich wiederholt, dass keine trüben Kolonien entstanden, sondern nur klare Kolonien, die in der Objektglasagglutination im Vi-Serum negativ reagierten. Trotzdem enthielten diese Kulturen aber das Vi-Antigen, da sie im Bindungsversuch ein Vi-Serum restlos erschöpften.

Dieses Phänomen: Anwesenheit des Vi-Antigens in Kulturen mit klaren Kolonien bei negativer Vi-Agglutination fand sich auch bei dem Coli-Stamme 6179/36 von *Gard*, der nur in dieser klaren Form vorlag. Die beiden anderen Coli-Stämme 1743/37 und 5396/38 wiesen dagegen deutlich den V-W-Formenwechsel auf, d. h. sie lagen in trüben und klaren Kolonien vor. Der Stamm 6179/36 ergab sowohl in der Objektglas- als auch in der Reagensglasagglutination in einem Vi-Serum eine negative Agglutination, war aber trotzdem imstande, das Vi-Agglutinin aus einem Typhus Vi-Serum völlig zu entfernen. Kontrollversuche mit anderen Coli-Kulturen sowie mit verschiedenen *Salmonella*-Kulturen wie *S. paratyphi* A, *S. paratyphi* B, *S. typhi* murium, *S. enteritidis*, *S. sendai* und der »Hirschfeld«-Kultur von *S. paratyphi* C, die in der W-Form vorliegt, ergaben ein negatives Resultat, d. h. diese Kulturen konnten das Vi-Agglutinin nicht binden. Man muss also damit rechnen, dass bestimmte Kulturen das Vi-Antigen in so geringer Menge oder derartig verteilt enthalten,

dass es nicht mit Hilfe der Agglutination, sondern nur mit Hilfe der Bindung nachweisbar ist. Immunisiert man mit einer derartigen lebenden Kultur, z. B. dem Coli-Stamme 6179/36, Kaninchen, so erhält man ein Serum mit Vi-Agglutininen, wodurch die Anwesenheit des Vi-Antigens in diesem Stamme ebenfalls bewiesen ist.

Beim V-W-Formenwechsel handelt es sich also — ebenso wie beim I- oder XII-Formenwechsel — um quantitative Antigen-Veränderungen, da alle Übergänge von der V-Form mit komplett entwickeltem Vi-Antigen über die VW-Form bis zur W-Form, der das Vi-Antigen völlig fehlt, vorkommen.

Da über die serologischen Eigenschaften des Vi-Antigens bei der Beschreibung von Typhus-Stämmen und der Ballerup-Kultur eingehend berichtet wurde, halte ich es für überflüssig, auf die Eigenschaften des Vi-Antigens bei diesen Coli-Stämmen näher einzugehen, sodass ich nur kurz das Wichtigste sagen will. Das Vi-Antigen dieser Kulturen ist mit dem Vi-Antigen von *S. typhi*, *S. paratyphi* C und *S. ballerup* serologisch identisch, wie es aus gekreuzten Absorptionsversuchen hervorgeht. Bei voller Entwicklung des Vi-Antigens in den V-Formen kommt es zur O-Inagglutinabilität, die durch Erhitzen auf 100° zerstört werden kann. Die W-Formen sind dagegen auch in lebendem Zustande gut O-agglutinabel. In den folgenden Tabellen sind einige Agglutinationsergebnisse zusammengestellt, aus denen die Anwesenheit des Vi-Antigens in den Coli-Kulturen hervorgeht.

Zwischen diesen Coli-Kulturen mit Vi-Antigen und *S. typhi* resp. *S. ballerup* bestehen keine O- und H-Antigenbeziehungen.

Zusammenfassend können wir also feststellen, dass die hier beschriebenen 4 Coli-Kulturen ausser dem bereits bekannt gewesenen Salmonella H-Antigen noch das Vi-Antigen enthalten, das dadurch bei folgenden Typen nachgewiesen ist: *S. typhi* (*A. Felix* und *R. M. Pitt*), *S. paratyphi* C (*F. Kauffmann*), *S. ballerup* (*F. Kauffmann* und *E. Moller*) und *S. coli* 1 (*F. Kauffmann*). (Betreffs der Nomenklatur dieses neuen Typus verweise ich auf die weiter unten folgende Besprechung der Ergebnisse).

Tabelle 1.
Vi-Serum von *S. typhi* (Vi-Titer 320).

Kultur	20	40	80	160	320	640	1280
<i>S. typhi</i> Watson V	++	++	++	++	+	±	—
<i>S. coli</i> 2624/36 V	++	++	++	+	±	—	—

Vi-Serum von *S. ballerup* (Vi-Titer 640).

Kultur	20	40	80	160	320	640	1280	2560
<i>S. typhi</i> Watson V	++	++	++	++	++	+	±	—
<i>S. coli</i> 2624/36 V	++	++	++	++	+	±	—	—

Vi-Serum von *S. coli* 2624/36 (Vi-Titer 640).

Kultur	20	40	80	160	320	640	1280	2560
<i>S. typhi</i> Watson V	++	++	++	++	+	+	±	—
<i>S. „ H 901 W</i>	—	—	—	—	—	—	—	—
<i>S. ballerup</i> V	++	++	++	++	+	±	—	—

Erläuterung zur Tabelle 1:

Zur Agglutination wurden lebende Agarkulturen in NaCl-Lösung aufgeschwemmt benutzt. Ablesung der Agglutination nach 2 Std. 37° Thermostat und weiteren 20 Std. Zimmertemperatur. ++, +, ± = verschiedene Stärke der Agglutination; ± = nur mit der Lupe 6× erkennbar. — = negative Agglutination.

II. Über Coli-Kulturen mit *Salmonella* H-Antigenen.

Es handelt sich hierbei um die soeben erwähnten 4 Coli-Kulturen mit Vi- + H-Antigen sowie um die beiden Coli-Kulturen 491/36 von *Gnosspeilus* und 5821/38 von *Gard*, die identisch sind und im Gegensatz zu den 4 ersten Kulturen kein

Vi-Antigen enthalten. In Bestätigung der Angaben von *Gross-pelius* sowie von *Gard* und *Eriksson* enthalten die beiden Kulturen 491/36 und 5821/38 *Salmonella* H-Antigen der unspezifischen Phase, das der 1,5... Phase nahe steht. Ich bezeichne diesen Typus mit »*Salmonella coli* 2«.

Sowohl das H-Antigen von *S. coli* 1 als auch dasjenige von *S. coli* 2 ist von der *Salmonella* 1,5... Phase (z. B. von *S. thompson* var. *berlin*) verschieden. Beide Phasen werden jedoch von dem diagnostischen »5-Serum« agglutiniert, das durch Absorption eines Serum von *S. thompson* var. *berlin* mit den Kulturen *S. anatum* unsp. und *S. newport* unsp. gewonnen ist. Auch untereinander sind die unspezifischen H-Antigene der beiden *Coli*-Typen 1 und 2 verschieden, wie es aus den folgenden Tabellen hervorgeht. In der Tabelle 2 sind die H-Agglutinationsergebnisse mit unabsorbierten H-Immunsereen dargestellt, während in der Tabelle 3 einige Absorptionsergebnisse, aus denen die Verschiedenheit dieser Kulturen hervorgeht, zusammengefasst sind.

Tabelle 2.
H-Agglutination.

Kulturen	H-Immunsereen			
	<i>S. coli</i> 1	<i>S. coli</i> 2	<i>S. thompson</i> unsp.	<i>S. paratyphi</i> B. unsp.
<i>S. paratyphi</i> B unsp.	100	100	400	12800
<i>S. newport</i> »	400	1600	800	6400
<i>S. thompson</i> »	6400	6400	6400	3200
<i>S. anatum</i> »	400	1600	1600	1600
<i>S. gaminara</i> »	50	400	100	3200
<i>S. kentucky</i> β	1600	1600	200	50
<i>S. coli</i> 1	6400	12800	6400	3200
<i>S. coli</i> 2	3200	25600	6400	400

Zeichenerklärung: Die Zahlen geben die H-Titer an.

Die H-Seren von *S. coli* 2 agglutinieren ausser den unspezifischen Kulturen noch die spezifische Phase von *S. london* (l, v) und die α -Phase von *S. dar es salaam* (l, w). Das H-Serum des Stammes 491/36 agglutiniert diese beiden Kulturen bis 1:40, das H-Serum von 5821/38 bis 1:160. Auch umgekehrt bestehen geringe übergreifende H-Agglutinationen.

Tabelle 3.
H-Absorption.

H-Immunsereen

Kulturen	S. coli 1			S. coli 2			S. thompson unsp.		
	nicht absorbiert	absorbiert mit		nicht absorbiert	absorbiert mit		nicht absorbiert	absorbiert mit	
		S.coli 2	Berlin		S.coli 1	Berlin		S.coli 1	S.coli 2
S. coli 1	×	×	×	×	—	—	×	—	×
S. coli 2	×	—	—	×	×	×	×	—	—
S. thompson unsp.	×	×	—	×	—	—	×	×	×

Zeichenerklärung:

× = positive H-Agglutination.

— = negative Agglutination.

Berlin = S. thompson unsp. (S. thompson var. berlin).

Drückt man die relativen H-Antigenbeziehungen dieser 3 Typen unabhängig vom Salmonella-Antigenschema aus, so lauten die Formeln von S. thompson unsp. = a, b, c, von S. coli 1 = a, b, e und von S. coli 2 = a, d.

Zusammenfassend können wir also sagen, dass ein zweiter Coli-Typus (S. coli 2) mit unspezifischem Salmonella H-Antigen vorliegt, das von dem H-Antigen des S. coli 1 Typus verschieden ist. Ausserdem unterscheiden sich diese beiden Typen durch ihre O-Antigene sowie durch ihre Vergärungsformeln, wie es aus der Tabelle 5 und der Kultur-Tabelle hervorgeht.

III. Über Coli-Kulturen mit Salmonella O-Antigenen.

Es handelt sich hierbei um 3 Kulturen »Zürich«, »Ka.« und »Zicht 11391«, die mit »Salmonella coli 3«, »Salmonella coli 4« und »Salmonella coli 5« bezeichnet werden, da sie 3 verschiedene serologische Typen der Salmonella-Gruppe sind.

1) *Salmonella coli* 3.

Der mit »Zürich« bezeichnete »Colistamm« dieses Typus wurde mir 1940 von *H. Schütze*, London mit der Angabe übersandt, dass diese ihm von *A. Grumbach*, Zürich, im Jahre 1933 geschickte Kultur, die Lactose spaltet, fast das ganze O-Antigen von *S. paratyphi* B enthalte. Auf Grund einer persönlichen Auskunft von *A. Grumbach* handelt es sich hierbei um folgendes:

»Der Stamm wurde aus dem Stuhl eines Patienten isoliert, der unter einem Paratyphus-ähnlichen Bild (sog. unspezifische Enteritis) erkrankt war. Die Blutkulturen waren negativ. Das Serum dagegen zeigte einen Widal mit Para B unter Mitagglutination von Breslau.... Irgend einen anderen der bekannten Darmpathogenen konnten wir nie isolieren.«

Auf Grund dieser Angaben besteht also die Wahrscheinlichkeit, dass die Kultur »Zürich« der Erreger einer Enteritis beim Menschen war, da sie zu einer positiven Widalreaktion gegenüber Stämmen mit dem IV. V-Antigen, das auch diese Kultur »Zürich« enthält, geführt hat.

Über das kulturelle Verhalten dieses Stammes gibt die folgende Kulturtabelle Auskunft. In serologischer Hinsicht kann ich die Angaben von *H. Schütze* bestätigen, dass dieser Stamm fast das ganze O-Antigen von *S. paratyphi* B besitzt. Er enthält die für *S. paratyphi* B charakteristischen O-Antigene IV. V., unterscheidet sich dagegen von diesem Typus durch das komplex gebaute XII-Antigen, dem der XII₂-Faktor fehlt.

Zum Verständnis dieser Befunde muss ich zunächst kurz auf die Serologie des XII-Antigens und den von mir gefundenen XII-Formenwechsel eingehen. (J. of Bacteriol. im Druck).

Das übergreifende XII-Antigen besteht aus mindestens 3 verschiedenen Partialantigenen XII₁, XII₂ und XII₃, von denen das XII₂-Antigen — in derselben Weise wie das I-Antigen — einem Formenwechsel unterworfen ist. Es kommen innerhalb desselben Stammes Kolonien mit stark entwickeltem XII₂-Antigen (++ Form), mässig entwickeltem XII₂-Antigen (+ Form) und schwach ent-

entwickeltem XII₂-Antigen (\pm Form) vor, die einander abspalten. Ausserdem ist die Verteilung der verschiedenen XII-Partialantigene bei den einzelnen Salmonella-Typen verschieden, wie es aus der folgenden Tabelle 4 hervorgeht.

Tabelle 4.

O-Antigenformeln unter Berücksichtigung der XII-Partialantigene.

Typ	O-Antigene
S. paratyphi A	[I]. II. XII ₁ . XII ₃ .
S. — „ var. durazzo	II. XII ₁ . XII ₃ .
S. paratyphi B	[I]. IV. V. XII ₁ [XII ₂].
S. reading	IV. XII ₁ . [XII ₂].
S. bredeney	[I]. IV. XXVII. XII ₁ . XII ₃ .
S. schleissheim	IV. XXVII. XII ₁ . XII ₃ .
S. abortus equi	IV. XII ₁ . XII ₃ .
S. abortus ovis	IV. XII ₁ . [XII ₂]. XII ₃ .
S. typhi T 4	IX. XII ₁ . XII ₂ . XII ₃ .
S. — T 2	IX. XII ₁ . XII ₃ .
S. enteritidis	[I]. IX. XII ₁ . [XII ₂]. XII ₃ .

Zeichenerklärung:

[] \approx diese Antigene sind einem Formelwechsel unterworfen, sodass \pm , + oder ++ Formen auftreten können.

Diese O-Antigenformeln sind nicht als vollständig zu betrachten, sondern in Wirklichkeit erheblich komplizierter; sie sollen nur die wesentlichsten Agglutinations- und Absorptionsergebnisse erklären.

Bei den in Tabelle 4 erwähnten Typhuskulturen T 2 und T 4 handelt es sich um die beiden von *Almon* und *Stovall* beschriebenen Kulturen, von denen die T 2 Kultur — in Bestätigung der Angaben von *Almon* und *Stovall* — ein O-Antigen weniger hat als die T 4 Kultur, die in ihrem O-Antigen dem bekannten Stamm 901 von *A. Felix* entspricht. Dieser Antigenverlust betrifft aber nicht, wie *Almon* und *Stovall* annahmen, das IX-Antigen, sondern das XII₂-Antigen. Innerhalb der Typhuskulturen mit vollständigem O-Antigen konnte bisher der XII-Formenwechsel nicht nachgewiesen werden, da z. B. sämtliche Kolonien des T 4- oder 901-Stammes das XII₂-Antigen stark entwickelt haben (++ Form). Es konnten also bisher nur Typhuskulturen mit oder ohne das XII₂-Antigen gefunden werden.

Bei dem Stamme »Zürich« konnte bisher weder das XII₂- noch das XII₃-Antigen nachgewiesen werden, sodass ich die Formel mit IV. V. XII..... bezeichne. Diese Kultur entspricht also in ihrem O-Antigen dem Chester-Typus (IV. V. XII...), auf dessen abweichendes XII-Antigen ich in meiner Arbeit über den XII-Formenwechsel bereits hingewiesen hatte. Dementsprechend konnte S. chester das O-Serum von »Zürich« restlos erschöpfen, wie auch umgekehrt der »Zürich«-Stamm das Chester O-Serum völlig erschöpfte. Dagegen führte die Absorption eines O-Serums von S. paratyphi B oder von S. reading mit S. coli 3 nicht zur völligen Erschöpfung dieser Seren, da noch die XII₂-Agglutinine zurückblieben. Zum Nachweis dieser XII₂-Agglutinine ist es nötig, Immunsere zu benutzen, die mit der XII++ Form hergestellt sind, sowie zur Agglutination Kulturen anzuwenden, die in der XII++ Form vorliegen. Um dieses zu erreichen, ist es notwendig, von geprüften Einzelkolonien auszugehen, die in der Objektglasagglutination mit einem starken XII₂-Serum (z. B. Ty 901) agglutiniert werden. So liegen z. B. von 20 Kolonien einer Paratyphus B-Kultur (originale Massenkultur) 2 Kolonien in der XII++ Form vor, während 18 Kolonien in der XII± Form vorliegen. Bei Fortzüchtung einer ++ Kolonie sind von 10 untersuchten Kolonien 9 Kolonien in der ++ Form und 1 Kolonie in der ± Form. Umgekehrt sind bei Fortzüchtung einer ± Kolonie von 10 Kolonien 9 Kolonien in der ± Form und 1 Kolonie in der ++ Form. (Der Prozentsatz der Abspaltung kann natürlich schwanken). Bei Kulturen, die sowohl das I-Antigen als auch das XII-Antigen besitzen, können beide Formenwechsel gleichzeitig vorkommen, sodass also 4 verschiedene serologische Formen innerhalb desselben Stammes vorkommen: I++, XII± Form, I++, XII++ Form, I±, XII± Form und I±, XII++ Form. Ohne Berücksichtigung dieser serologischen Variation, des »O-Formenwechsels«, sind Agglutinationsversuche dem Zufall überlassen und müssen zu widersprechenden Resultaten führen und zwar in ähnlicher Weise wie beim Phasenwechsel innerhalb der H-Antigene, wenn man diesen Phasenwechsel nicht berücksichtigt.

Durch Absorption des O-Serums von »Zürich« mit S. reading konnte das V-Agglutinin nachgewiesen werden, ebenso wie auch die »Zürich«-Kultur in einem aus einem Paratyphus B-Serum gewonnenen V-Serum prompt agglutinierte. Das Vi-Antigen konnte bei der »Zürich«-Kultur nicht nachgewiesen werden. Ein H-Serum der »Zürich«-Kultur liess alle Salmonella-Kulturen unbeeinflusst, wie auch die »Zürich«-Kultur von keinem der Salmonella H-Seren agglutiniert wurde. Es wurden keine Versuche unternommen, durch H-Immunsersumzusatz künstlich einen Phasenwechsel bei der »Zürich«-Kultur herbeizuführen.

Zusammenfassend lässt sich also über diesen »Zürich«-Stamm sagen, dass er die Haupt O-Antigene der Salmonella B-Gruppe IV. V. besitzt, dass er sich aber im Bau des XII-Antigens von S. paratyphi B und anderen Typen dieser Gruppe unterscheidet. Er stimmt hinsichtlich seines O-Antigens mit S. chester überein, sodass die O-Formel mit IV. V. XII... angegeben werden kann.

Da es sich bei diesem Typus »S. coli 3« wahrscheinlich um einen Enteritiserreger handelt, der prompt Lactose spaltet, so wird die Unzulänglichkeit und Einseitigkeit unserer Routinediagnostik, die mit lactosehaltigen Platten arbeitet und alle Lactosevergärer als »banal« ausschaltet, klar. Auf die praktischen Folgerungen dieser Befunde will ich jedoch in diesem Zusammenhange nicht näher eingehen. Ich möchte nur darauf hinweisen, dass dem Nachweise von Salmonella O-Antigenen in Coli-Kulturen möglicherweise besondere Bedeutung in pathogenetischer Hinsicht zukommt. Zur Klärung dieser Frage sind jedoch systematische serologische und klinische Untersuchungen notwendig. Die Diagnose »Coli« als solche muss als völlig unzureichend bezeichnet werden, da nur eine exakte *Typendiagnose* auf Grund serologischer und kultureller Untersuchungen für ätiologische und epidemiologische Zwecke Bedeutung hat.

2) *Salmonella coli* 4.

Der mit »Ka.« bezeichnete »Colistamm« wurde mir 1940 von N. Černozubov, Zagreb mit der Angabe übersandt, dass

diese von A. *Hupbauer* herrührende Kultur (Karagjorgjevo) die I.IV-Antigene enthalte. Auf Grund einer persönlichen Auskunft von A. *Hupbauer*, Zagreb, handelt es sich hierbei um folgendes:

»Dieser Stamm wurde aus dem Mageninhalt von vier Monate alten, abortierten Schweineföten gezüchtet. Diese wurden dem Institut am 18. April 1927 zugestellt. Auf dem Gestüt Karagjorgjevo verwarfen mehrere Zuchttiere der dortigen Herde. Auf dem Gestüte herrschte damals, so wie auch einige Jahre vorher Paratyphus-abort bei den Stuten. Bei der Sektion der Föten wurden Blutungen am Herzmuskel und serösen Häuten sowie ein Milztumor festgestellt. Das aus dem Mageninhalt in Reinkultur herausgezüchtete Bakterium war sehr beweglich, veränderte Endo nicht, bildete Indol nicht, jedoch Schwefelwasserstoff. Maltose und Glukose wurden gespalten, Saccharose nicht. Laktose wurde kaum merklich gerötet. Nachdem der Stamm etwa ein Jahr lang im Laboratorium aufgehoben war, wurde auch Laktose gespalten mit Gasbildung. Milch wurde nicht zur Gerinnung gebracht, Mäuse starben nicht, verwarfen jedoch am 6.—8. Tage. Lakmus wurde gerötet, später gebläut.

Der Stamm wurde durch B-Serum stark agglutiniert. Durch Seren hergestellt mittels Hyperimmunisierung mit Stutenabortstämmen wurde dieser Stamm stark agglutiniert (1:1600 +).

Der Stamm wurde irrtümlicherweise mit Rücksicht auf seine serologischen Eigenschaften und die anfänglich nur sehr schwache Änderung der Laktose als Paratyphus bezeichnet. Als er später Laktose angriff, wurde der Fehler eingesehen.«

Das kulturelle Verhalten dieses Stammes »Ka.« ist in der Kulturtafel wiedergegeben. Über das serologische Verhalten ist zu sagen, dass dieser Stamm die Haupt O-Antigene von *S. schleissheim* IV.XXVII. enthält und imstande ist, ein O-Serum von *S. schleissheim* völlig zu erschöpfen. Ausserdem besitzt der Stamm »Ka.« ein auf *S. london* übergreifendes O-Antigen, das in *S. schleissheim* anscheinend fehlt, aber noch nicht näher analysiert wurde. Möglicherweise liegt hier ein O-Formenwechsel vor, sodass dieses übergreifende Antigen auch in Kulturen von *S. schleissheim* vorkommen kann. Ich will daher die O-Formel des Stammes »Ka.« vorläufig mit IV.XXVII.XII... angeben. Das H-Antigen des Stammes »Ka.« hat zu keinem der bekannten *Salmonella* H-Antigene Be-

ziehungen und ist auch von dem H-Antigen des Stammes »Zürich« sowie der anderen hier beschriebenen Coli-Stämme verschieden (siehe Tabelle 5).

Zusammenfassend lässt sich also über diesen »Ka.«-Stamm sagen, dass er die Haupt O-Antigene von *S. schleissheim* IV. XXVII besitzt. Es ist auffallend, dass 3 verschiedene Typen, welche die IV. XXVII-Antigene enthalten, nämlich *S. abortus bovis*, *S. schleissheim* und dieser neue Typus *S. coli* 4, bei Tieren gefunden wurden und zwar 2 dieser Typen bei Abort. Auch bei *S. schleissheim* handelt es sich möglicherweise um einen Aborterreger, da die originale Kultur aus einer Kuh isoliert wurde. Bekanntlich wurde *S. schleissheim* durch *E. Boecker* und *K. Dzionara* auch beim Menschen als Enteritis-erreger nachgewiesen, da ich (1940) feststellen konnte, dass der von *E. Boecker* und *K. Dzionara* beschriebene Stamm zu *S. schleissheim* gehört.

3) *Salmonella coli* 5.

Der mit »Zicht 11391« bezeichnete Stamm wurde mir Ende 1939 von *F. Schiff*, New York, zur Begutachtung übersandt, der hierüber mit *I. Saphra* näher berichten wird. Ich will mich hier daher nur darauf beschränken, die Befunde zu bestätigen, nach denen die Kultur »Zicht 11391« das gesamte O-Antigen von *S. onderstepoort* (I). VI. XIV. XXV. enthält, während das H-Antigen keine Beziehungen zur *Salmonella*-Gruppe besitzt. Das kulturelle Verhalten dieses Stammes geht aus der folgenden Kulturtabelle hervor.

Aus der Kulturtabelle geht hervor, dass die 9 untersuchten Coli-Kulturen zu 5 verschiedenen Vergärungstypen gehören, die ebenso vielen Serotypen entsprechen. Zu *S. coli* 1 gehören 4 Kulturen, von denen 1 aus Göteborg und 3 aus Stockholm stammen. Zu *S. coli* 2 gehören 2 Kulturen und zwar 1 aus Göteborg und 1 aus Stockholm. Man ersieht hieraus, dass man denselben kulturellen und serologischen Typus an verschiedenen Stellen wiederfinden kann.

Lactose wird von 8 Kulturen prompt gespalten, während der letzte Stamm die Lactose verzögert angreift. Nur der 1.

Kulturtabelle.

	<i>S. coli</i> 1. 2624/36 6179/36 1743/37 5396/38	<i>S. coli</i> 2. 491/36 5821/38	<i>S. coli</i> 3. „Zürich“	<i>S. coli</i> 4. „Ka.“	<i>S. coli</i> 5. „Zicht 11391“
Salmonella-Antigene	H + Vi	H	O	O	O
Adonit	— ₃₀	— ₃₀	— ₃₀	— ₃₀	— ₃₀
Arabinose, Dextrin, Dextrose, Maltose, Mannit, Rhamnose, Sorbit, Trehalose, Xylose	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹
Dulcit	— ₃₀	+ ¹	— ₃₀	— ₃₀	— ₃₀
Inosit	— ₃₀	— ₃₀	+ ²	— ₃₀	— ₃₀
Lactose	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹
Saccharose	— ₃₀	—od.—+	+ ²	+ ¹	+ ¹
Salicin	—+	—+	—+	—+	—+
Indol	+	—	—	—	—
H ₂ S	+	+	+	+	—
Gelatine	—	—	—	—	+
Simmons Agar mit Arabinose, Dextrose, Na-Citrat u. Rhamnose	+	+	+	+	+
Simmons Agar mit Dulcit	—	+	—	—	—
d-Tartrat	+ ⁵⁻⁶	+ ⁵⁻⁶	+ ⁶	+ ⁷	— ¹⁴
l- „	—×	— ¹⁴	+ ⁶	—×	— ¹⁴
i- „	— ¹⁴	— ¹⁴	— ¹⁴	— ¹⁴	— ¹⁴
Na-Citrat	+ ¹⁻²	+ ²	+ ²	+ ²	—×
Mukat	+ ¹	+ ¹	+ ¹	— ¹⁴	+ ¹
Brillantgrünagar	grün	grün	grün	grün	grün

Zeichenerklärung:

—₃₀ = negativ nach 30 Tagen.+¹ = positiv nach 1 Tage.

—+ = spät positiv.

—× = unregelmässig, meist negativ.

Die Vergärung von l-Tartrat scheint bei diesen Coli-Kulturen unregelmässig, meist negativ zu sein.

Typ bildet Indol, und nur der 5. Typ verflüssigt langsam die Gelatine, bildet aber im Gegensatz zu den anderen Typen kein H_2S .

Über die Herkunft der Stockholmer Stämme, die anscheinend in keinem Zusammenhange mit menschlichen Erkrankungen stehen, sind in der Arbeit von *S. Gard* und *E. J. Eriks-son* nähere Angaben gemacht worden. Diese Kulturen sowie die beiden Kulturen von *Gnosselius*, also sämtliche Stämme von *S. coli* 1 und 2, wurden mit Hilfe des *Wassén*-Verfahrens isoliert und zwar dadurch, dass sie von einem unspezifischen *Salmonella* H-Serum beeinflusst wurden. Im Gegensatz zu diesen Kulturen enthalten die 3 anderen Typen *S. coli* 3, 4 und 5 nur *Salmonella* O-Antigene. Das Vi-Antigen wurde nur bei

Tabelle 5.

Typ	O-Antigen	H-Antigen		Vi-Antigen
		1. Phase	2. Phase	
<i>S. paratyphi</i> B	[I]. IV. V. XII...	b	1,2...	—
<i>S. coli</i> 3	IV. V. XII...	z_{20}	.	—
<i>S. schleissheim</i>	IV. XXVII. XII...	b, z_{12}	—	—
<i>S. coli</i> 4	IV. [XXXVII.] XII...	z_{21}	.	—
<i>S. paratyphi</i> C	VI. VII. XII...	c	1,5...	A
<i>S. typhi</i>	IX. XII...	d	—	A
<i>S. onderstepoort</i>	(I). VI. XIV. XXV.	e, h	1,5...	—
<i>S. coli</i> 5	(I). VI. XIV. XXV.	z_{22}	.	—
<i>S. ballerup</i>	XXIX.	z_{14}	—	A
<i>S. coli</i> 1	XXXI.	.	1,5...	A
<i>S. coli</i> 2	XXXII.	.	1,5...	—

Zeichenerklärung:

[] = Diese Antigene können auch fehlen.

(I) = Es ist nur ein Teil des I-Antigens vorhanden.

XII ... = Die Partialantigene des XII-Antigens sind hier nicht berücksichtigt.

. = nicht näher untersucht.

— = fehlt.

den 4 Kulturen von *S. coli* 1 nachgewiesen, die gleichzeitig unspezifisches H-Antigen besitzen.

In der Tabelle 5 sind die Antigenformeln der hier untersuchten Coli-Kulturen im Vergleich zu verschiedenen, serologisch verwandten *Salmonella*-Typen zusammengestellt. Der Phasenwechsel der Coli-Kulturen ist von mir noch nicht näher untersucht worden, sodass die Formeln nicht als vollständig zu betrachten sind. In der Objektglasagglutination reagierten alle untersuchten Coli-Kolonien der Typen *S. coli* 1 und 2 in einem Berlin H.-Serum (1,5...) positiv. Es muss jedoch darauf hingewiesen werden, dass die in der Tabelle 5 mit 1,5... bezeichneten Phasen nicht unter einander identisch sind. Es kam mir in dieser Arbeit vorläufig nur darauf an, nachzuweisen, dass die beiden Typen *S. coli* 1 und 2 unspezifisches H-Antigen enthalten, wodurch die Angaben von *Gnosselius*, *Gard* und *Eriksson* bestätigt sind.

Besprechung der Ergebnisse.

Aus den vorliegenden Untersuchungen geht hervor, dass bestimmte für *Salmonella*-Kulturen charakteristische O-, H- und Vi-Antigene auch bei verschiedenen Coli-Kulturen nachgewiesen sind. Es folgt hieraus, dass zwischen der sogenannten »*Salmonella*-Gruppe« und der sogenannten »*Coli*-Gruppe« in serologischer Hinsicht kein prinzipieller Unterschied besteht, also auch keine scharfe Grenze! Schon früher (1937) hatte ich anlässlich des Befundes einer lactosespaltenden *Salmonella*-Variante darauf hingewiesen, dass auch in kultureller Hinsicht zwischen der *Salmonella*- und *Coli*-Gruppe eine solche Grenze fehlt, zumal auch indolpositive und gelatineverflüssigende *Salmonella*-Typen bekannt sind. Ich hatte daher folgende, serologisch orientierte *Salmonella*-Definition gegeben:

»Salmonellabakterien sind gramnegative Bakterien, die auf Grund ihrer Antigenstruktur in das Kauffmann-White-Schema eingefügt werden können.«

Die neuen, hier mitgeteilten Befunde von *Coli*-Stämmen

mit typischen *Salmonella*-Antigenen haben die Zweckmässigkeit und Notwendigkeit einer solchen serologischen *Salmonella*-Definition bestätigt und alle noch hiergegen bestehenden Bedenken zerstreut. Wir können nämlich die Schwierigkeiten und Unstimmigkeiten, die bei einer kulturell und serologisch orientierten Definition auftreten, nur dann umgehen, wenn wir den *Salmonella*-Begriff *serologisch* fassen.

Wir verstehen also — entsprechend der obigen Definition — unter der *Salmonella*-Gruppe eine serologisch zusammengehörige Gruppe verschiedener Typen, unabhängig davon, wie sich diese Typen in kultureller oder pathogenetischer Hinsicht verhalten. Wir bezeichnen also alle diejenigen Typen gramnegativer Bakterien als *Salmonella*-Typen, die auf Grund ihrer Antigenstruktur in das *Kauffmann-White-Schema* eingefügt werden können. Deshalb müssen auch die hier beschriebenen 5 *Coli*-Typen mit *Salmonella*-Antigenen als *Salmonella*-Typen bezeichnet werden, obwohl sie sich kulturell wie *Colistämme* verhalten. Um diese Doppelstellung zu kennzeichnen, nenne ich diese Typen »*Salmonella coli*«, sodass sie serologisch auf gleicher Stufe mit »*Salmonella typhi*«, »*Salmonella paratyphi*«, »*Salmonella enteritidis*« u. s. w. stehen.

Da hierdurch das Wort »*Salmonella*-Gruppe« nicht mehr auf bestimmte Typen mit besonderer Vergärungsformel oder Pathogenität beschränkt ist, sondern darüber hinaus alle serologisch zusammengehörigen Typen der gramnegativen Bakterien umfasst, so gebrauchen wir für die bisherige, engere *Salmonella*-Gruppe eine andere Bezeichnung, die wohl am besten »*Typhus-Paratyphus-Enteritis-Gruppe*« lautet, zumal dieser Name in der Literatur gebräuchlich ist.

Die Einführung des umfassenden serologischen Begriffes »*Salmonella*-Gruppe« zwingt uns nämlich keineswegs dazu, die früheren Begriffe »*Coli*-Gruppe«, »*Typhus-Paratyphus-Enteritis-Gruppe*« u. a. aufzugeben, sodass diese besonders mit Rücksicht auf die medizinische Praxis beibehalten werden können. Wir müssen uns nur bewusst bleiben, dass diese Gruppen unscharf definiert und schlecht von einander abgegrenzt sind. Tatsächlich können wir heute nur einzelne Typen auf

Grund kultureller, serologischer und pathogenetischer Merkmale klar definieren; wir sind dagegen nicht in der Lage, genaue Gruppendifinitionen zu geben, und werden es wohl auch niemals können, weil es eben in der Natur keine scharf abgetrennten Bakteriengruppen gibt. Die zukünftige Forschung muss also in erster Linie eine *Typenforschung* sein, zumal diese in ätiologischer und epidemiologischer Hinsicht die entscheidende Rolle spielt.

Es lässt sich im heutigen Stadium der Forschung nicht übersehen, wo später die Grenzen der Salmonella-Gruppe liegen werden, d. h. welche Typen gramnegativer Bakterien mit bestimmter Antigenstruktur einst von ihr umschlossen werden. In diesem Zusammenhange sei an die Untersuchungen von H. Schütze über die serologischen Beziehungen der Pseudotuberkulose-Typen II A und II B zum IV-Antigen der Salmonella-Gruppe erinnert, die von F. Kauffmann (1932) näher analysiert wurden.

Es ist möglich, dass die weitere Forschung über die gramnegativen Bakterien hinausgehen wird, wenn nämlich auch grampositive Bakterien oder Kokken in das *Kauffmann-White-Schema* eingefügt werden können. Wir würden dann zu einem umfassenden *Antigenschema* gelangen, das — chemischen Strukturformeln entsprechend — die serologischen Beziehungen der betreffenden Keime klarlegt. Die Salmonella-Gruppe wäre dann nur ein Teil dieses Antigenschemas, also gewissermassen ihr Kristallisationscentrum.

Auf Grund dieser Auffassung, dass die Antigenstruktur der Bakterien ein Mosaik darstellt, das aus bestimmten, immer wiederkehrenden Bausteinen besteht, ist der eingangs erwähnte Begriff der »Paragglutination« hinfällig geworden. Zwischen »Paragglutination« und »Agglutination« besteht nämlich überhaupt kein Unterschied, da beide dieselben spezifischen Immunitätsvorgänge sind. Die Tatsache, dass bestimmte Colistämme von Typhus- oder Dysenterie-Seren agglutiniert werden, ist keineswegs durch die völlig haltlose Hypothese zu erklären, dass diese Colistämme im Darm des Kranken die betreffenden Antigene erworben haben, sondern

einfach dadurch, dass sie diese Antigene bereits hatten. Dass eine solche »Paragglutination« bei weiterer Fortzüchtung der betreffenden Kulturen verschwinden kann, ist auf Grund unserer heutigen Kenntnisse kein Rätsel mehr, da bestimmte Antigene einem Formenwechsel unterworfen sind, verloren gehen oder in Rauh-Antigene umgewandelt werden können.

Der Begriff »Paragglutination« ist also fallen zu lassen, da es sich hierbei um nichts anderes als um eine Agglutination handelt und zwar auf Grund von Antigenen, die in den betreffenden Keimen bereits vorhanden waren.

Zusammenfassung.

- 1) Es wird über verschiedene Coli-Kulturen berichtet, die *Salmonella*-Antigene besitzen und zwar teils Vi- und H-Antigene, teils H-Antigene oder teils O-Antigene.
- 2) Bei 3 Coli-Kulturen mit Vi-Antigen wurde der V-W-Formenwechsel nachgewiesen.
- 3) Das Vi-Antigen der Coli-Kulturen ist mit dem Vi-Antigen von *S. typhi*, *S. paratyphi* C und *S. ballerup* serologisch identisch.
- 4) Bei einem vierten Coli-Stamme, der eine negative Vi-Agglutination ergab, konnte das Vi-Antigen durch den Bindungsversuch nachgewiesen werden.
- 5) Die hier beschriebenen Coli-Typen werden mit »*Salmonella coli* 1, 2, 3, 4 oder 5« bezeichnet und in einer Antigentabelle mit verwandten *Salmonella*-Typen zusammengestellt.
- 6) Es wird die Notwendigkeit einer serologischen *Salmonella*-Definition: »*Salmonella*-Bakterien sind gramnegative Bakterien, die auf Grund ihrer Antigenstruktur in das *Kauffmann-White-Schema* eingefügt werden können«, betont.
- 7) Durch die Einführung dieses übergeordneten serologischen »*Salmonella*«-Begriffes wird die Einteilung dieser grossen Gruppe gramnegativer Bakterien in mehrere Un-

tergruppen: »Coli-Gruppe«, »Typhus-Paratyphus-Enteritis-Gruppe« u. s. w. nicht verhindert.

- 8) Es wird auf die Notwendigkeit einer weiteren, vor allem serologisch orientierten *Typenforschung* hingewiesen, da wir nur in der Lage sind, die einzelnen *Typen* scharf von einander abzugrenzen, nicht dagegen die einzelnen Gruppen, deren Definition mehr oder weniger willkürlich bleiben muss.
- 9) Es wird über die nähere Analyse des XII-Antigens und den »XII-Formenwechsel« berichtet.
- 10) Der Begriff »*Paragglutination*« ist fallen zu lassen, da es sich hierbei um nichts anderes als um eine »*Agglutination*« handelt und zwar auf Grund von Antigenen, die in den betreffenden Keimen bereits vorhanden waren.

Inzwischen erhielt ich von *H. Braun*, *W. Silberstein* und *N. Uelker* eine Coli-Kultur »B« mit der Angabe, dass diese Kultur, die unter dem Namen »Colibazillus pseudosalmonella B Istanbul« beschrieben wurde, die IV. XII-Antigene enthalte. Ich kann diese Angaben bestätigen und dahin ergänzen, dass die Kultur »B« nicht das ganze XII-Antigen von *S. reading* enthält, da der XII₂-Faktor fehlt. Das H-Antigen ist mit dem H-Antigen von *S. coli* 4 (IV. XXVII. XII. . . z₂₁) identisch, so dass die Antigenformel also IV. XII. . . z₂₁ lautet. Wahrscheinlich handelt es sich bei dem Stamme »B« um eine Verlustvariante von *S. coli* 4, zumal auch das kulturelle Verhalten — mit Ausnahme von Mukat — dasselbe ist.

Bei dieser Gelegenheit sei erwähnt, dass einem von *E. Hormaeche* erhaltenen Bredeney-Stamme (25/1) das XXVII-Antigen fehlt, sodass hier also eine Parallele zu dem Stamme »B« vorliegt.

Ferner erhielt ich von *W. Silberstein* eine Kultur »Ali« und von *J. Hohn* eine Kultur Nr. 9988 mit der Angabe, dass diese Kulturen vom VI. VII-Serum stark agglutiniert wurden. Ich konnte dieses bestätigen und fand, dass sich beide Kulturen von einander unterscheiden, und dass ihre O-Antigene nicht mit dem VI. VII-Antigen identisch, sondern nur nahe verwandt sind.

Bei der Kultur 9988 wurden Kolonien festgestellt, die nicht vom VI VII-Serum agglutiniert wurden, worüber später in einer besonderen Arbeit berichtet werden soll.

Kürzlich konnte ich aus den Faeces eines 6 Monate alten Kindes (N), das an chronischer Dyspepsie litt, einen Coli-Stamm isolieren, der von mehreren Salmonella O-Seren mit VI-Antikörpern agglutiniert wurde, aber von den oben erwähnten Kulturen »Ali« und 9988 verschieden war.

Ausserdem wurde von J. Hohn eine weitere Coli-Kultur Nr. 3570 mit der Angabe übersandt, dass sie unspezifisches Salmonella H-Antigen (1,5...) enthalte. Eine orientierende Untersuchung bestätigte diese Feststellung und ergab, dass hier ein neuer von S. coli 1 und S. coli 2 verschiedener Typus vorliegt.

Aus diesen Befunden geht klar hervor, dass es sich bei derartigen Coli-Kulturen mit Salmonella-Antigenen nicht um Ausnahmen handeln kann, sondern um Angehörige einer grossen »*Salmonella coli-Gruppe*«, die aus zahlreichen serologisch und kulturell verschiedenen Typen besteht.

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EXPERIMENTAL STUDIES ON THE NORMAL AND PATHOLOGICAL HISTOPHYSIOLOGY OF THE PROSTATE GLAND. III.

ON THE PRESENCE OF LIPOIDS IN THE PROSTATIC EPITHELIUM.

By *P. Gylling*.

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I am greatly indebted to the directors pro tem. of the Anatomical Institute, Doctors Göran Hjelmman and Niilo Pesonen, for the kind interest they have always shown in my work. In this connection I especially want to thank Professor Axel Wallgren for the valuable help he has given me. My thanks are also due to Doctors Martti J. Mustakallio and Johannes Wahlberg.

During my earlier studies of the histophysiology of the prostatic epithelium I noticed, in different preparations, formations which could not be directly related to the intracellular phenomena associated with the production of secretion. In a number of osmiumstained preparations these drop-resembling formations are specially prominent. *Moore, Price* and *Gallagher* (1930) also observed similar greenish or grey drops in the prostatic epithelium of castrated rats after the preparations had been fixed in osmium. The above-mentioned authors said they were not wholly clear on the nature and significance of these drops. In the present investigation preliminary experiments have shown that such preparations as were fixed and stained according to current methods for fat examination, in certain cases presented clear fat drops in the

same cellular regions as were described by the above-mentioned writers. In earlier investigations, lipoids were occasionally observed in the prostatic epithelium of man and in certain animal species. On the basis of these data and of the findings yielded in preliminary experiments, the following questions were formulated:

1. Is it possible to observe lipoids in the prostatic epithelium of the normal full-grown rabbit.
2. In that case, is the presence of lipoids dependent on the cellular activity in the prostatic epithelium.
3. Is it possible to observe any change in the fat content of the epithelial cells during the atrophy of the prostate which sets in if the stimulating effect of the testicular hormone on the prostate gland ceases.
4. Are the lipoids singly or doubly refractive in polarized light.

Material. In the present experiments 30 male rabbits in all were used. With regard to the selection of the test animals and their preparatory treatment the reader is referred to p. 40, vol XVIII, I, of this journal. According to *Romeis* (1926), the lipoids in the prostatic epithelium of man are subject to changes which are dependent on age. As the present experiments refer exclusively to fully grown test animals, the age of the animals was standardized at about 1 year. The picture of the cellular activity of the prostatic epithelium which forms the basis of this investigation, is built on the experiences made in two earlier investigations into the histophysiology of the prostatic epithelium (see this journal vol XVIII, 1).

The prostatic epithelium of normal test animals was examined during stages of comparatively low cellular activity, a) after the rabbits had been living in complete isolation from other animals for months, b) when their cages had then been surrounded by oestral females for a couple of days, and c) after coitus interruptus. During different stages of marked cellular activity, the prostatic epithelium was examined 15 seconds, 3 minutes, 6 minutes, 9 minutes, 14 minutes, and 20 minutes after ejaculation. 2 rabbits per stage were used in these 9 experimental stages. The cellular activity in the prostatic epithelium was chiefly characterized by production of secretion. — The various degrees of epithelial atrophy were observed in an examination of the prostate gland after the

rabbits had been castrated for 5 days, 10 days, 20 days and 60 days. 3 rabbits were used in each of these 4 experimental stages.

Technique. By far the best and most reliable preparations were obtained, when the tissue pieces were cut out and frozen in carbonic acid snow immediately after the animal had been chloroformed. The piece of tissue was not allowed to thaw before sections were cut with a freezing microtome and fixed according to *Schultz-Braun's* method. Fairly good preparations were also obtained when small bits of the gland were fixed for 5—6 hours in a 10 per cent formol or Orth's solution. By the first-mentioned method the sections stuck without difficulty directly to a dry object-glass. After fixation in the two last-mentioned solutions it was advisable to dip the sections in thymol water and then attach them to an object-glass which had been smeared with levulose syrup. After all these fixations, sections were stained with Sudan III according to *Daddi*, with Sehalaeh R according to *Michaelis* and with Sudan-Orange according to *Romeis*. The nuclei were contrast-stained with hemalun or methylen blue. In preparations made for the study of chondriome or Golgi apparatus in the cells of the prostatic epithelium, the fat was sometimes very well preserved, presenting opportunities for comparative studies of the mutual relation of the different cell constituents. — The refractive power of the fatty substances was examined in an ordinary Zeiss polarization microscope.

INVESTIGATIONS.

Normal test animals. Table 1 shows the presence of fat in normal rabbits. In the first column of the table are entered the results obtained by Schultz-Braun's method. The second column includes the preparations fixed in formol, and the third column the preparations which were fixed in Orth's solution. The small columns indicate the staining methods. III means Sudan III, R means Sehalaeh R, and R₁ Sudan-Orange according to *Romeis*. Is. denotes preparations from test animals which have long been isolated, sex. preparations from a male rabbit which has been kept in the neighbourhood of oestral females, ci. denotes preparations made after coitus interruptus. The figures entered below the above terms denote the times, expressed in seconds or minutes, which have elapsed after the ejaculation up to the chloroforming of the test animals. The presence of fat is denoted by +, the absence of fat by —. The signs in the same horizontal line refer to the same gland and are the results of controls of the same gland by different methods.

The table shows that the prostatic epithelium of normal rabbits does not usually contain fat. Only in 3 out of 18 rabbits did the

Experiment stage	Schultz-Brauns			Formol			Orth		
	III	R	R ₁	III	R	R ₁	III	R	R ₁
is	=	=	=	=	=	=	=	=	=
sex	±	±	±?	±? ±?	=		±? ±?	=	
ci	=	=	=	=	=	=	=	=	=
15 sec.	=	=	=	=	=	=	=	=	=
3 min.	=	=	=	=	=	=	=	=	=
6 min.	=	=	=	=	=	=	=	=	=
9 min.	=	=	=	=	=	=	=	=	=
14 min.	±	±	=	±	=	=	=	=	=
20 min.	±	±	=	±	±	=	=	=	=

Table 1.

prostatic epithelium contain fat. The fact that fat was mainly observed by the Schultz-Braun method no doubt is due to the superiority of this method.

In spite of the fact that the fat occurred in somewhat different quantities in the prostatic epithelium of these 3 rabbits, its localization was on the whole identical. The epithelial cells in the first of these rabbits contained few and very small fat drops, the majority of which were situated just at the base of the cell, between the basally placed cell nucleus and the cell wall. In a fair number of cases, small internuclear fat drops were also seen. Fat drops were very rarely observed in the apical half of the cell.

An abundant amount of fat was found in the prostatic epithelium of the rabbit which was chloroformed 14 min. after ejaculation. Large conglomerations of fat drops occurred in the most basal parts of the epithelial cells and also around, and especially lateral of, the basally situated cell nucleus. Fat drops were only in exceptional cases found in the apical portions of the cells. Isolated little drops of fat could also be observed in the connective tissue lying close to the epithelium.

The fat content of the prostatic epithelium in the third of these rabbits lay halfway between the content of lipoid in the prostatic epithelia of the two first-mentioned rabbits. Golgi and chondriome preparations seemed to show that the fat drops were not directly related to the chondriome or the Golgi apparatus.

Fat drops were in no case observed in the lumina of these prostatic glands.

The fat drops described above were all singly refractive in polarized light.



Plate 1.

Marked cellular activity in normal prostatic epithelium. No fat drops. *Schultz-Braun*. Sudan III. Enlargement 166 X.

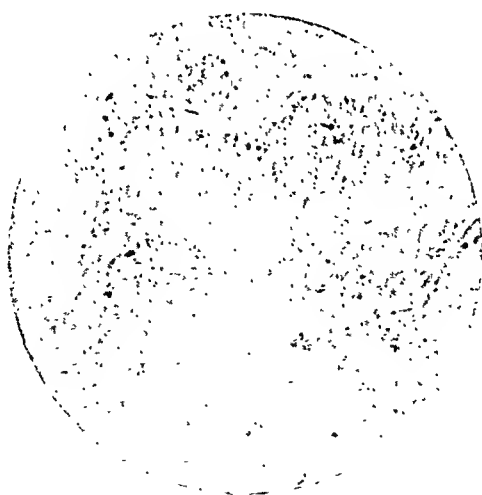


Plate 2.

Incipient epithelial atrophy. The small dark granules are fat drops. *Schultz-Braun*. Sudan III. Enlargement 166 X.

Castrated test animals. Table 2 shows the presence of fat in the prostatic epithelium of castrated test animals. The table is arranged like table 1. The figures denote the number of days during which the test animals were castrated. The preparations which were made at unequal intervals after bilateral castration present more regular

Experiment stage	Schultz-Brauns			Formol			Orth		
	III	R	R ₁	III	R	R ₁	III	R	R ₁
C 5	+	+	+	+	+	+	+	+	+
	—	—	—	—	—	—	—	—	—
	+	+	+	+	+	+	+	+	+
C 10	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+
C 20	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+
C 60	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+
	+	+	+	+	+	+	+	+	+

Table 2.

values for the fat content of the prostatic epithelium than the preparations taken from normal test animals. The table also shows that the results obtained with different methods of fixation and staining now better agree with one another.

All the drops of lipid which occurred in the epithelium after castration were singly refractive in polarized light.

An account will be given below of fat conditions in the prostatic epithelium after castration and each experimental stage will be described separately.

Preparations taken 5 days after castration. In one of the test animals no fat could be observed in the prostatic epithelium. — The prostatic epithelia of the other two test animals, on the other hand, closely resembled each other with regard to fat. Fat occurred very sparsely in these epithelia. No fat whatever was observable in some cells. In other cells fat, in the form of one or several small drops, was visible. These drops were usually found basally in the cell between the cell wall and the nucleus. Occasionally, a drop

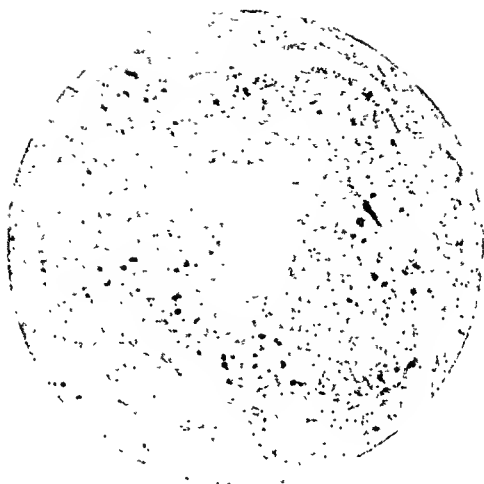


Plate 3.

Fairly clear epithelial atrophy. The small dark granules are fat drops. *Schultz-Braun*. Sudan III. Enlargement 166 X.

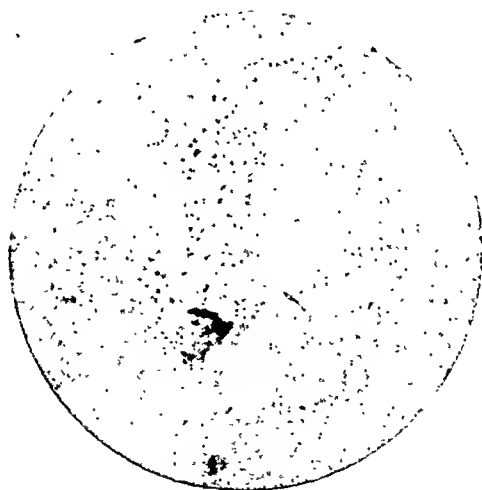


Plate 4.

Clear epithelial atrophy. The small dark granules are fat drops. *Schultz-Braun*. Sudan III. Enlargement 166 X.

occurred in a somewhat more apical position. No fat drops were found in the apical half of the epithelial cells.

In the connective tissue lying close to the epithelium small fat drops might be seen in exceptional cases. No fat drops occurred in the glandular lumen.

Preparations taken 10 days after castration. In this stage fat

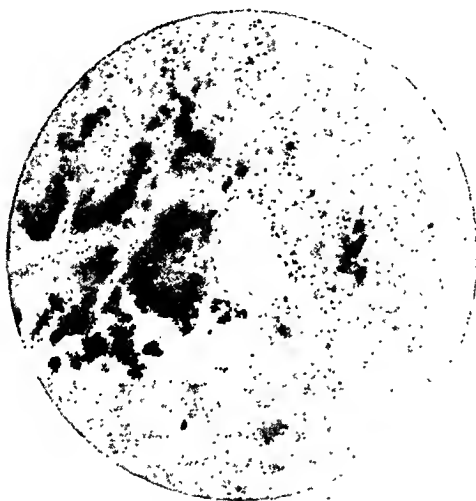


Plate 5.

Advanced epithelial atrophy. The small dark granules are fat drops.
Schultz-Braun. Sudan III. Enlargement 166 X.

could be observed in all three prostatic epithelia. The fat drops were as a rule somewhat larger than in the preceding stage. They were also more numerous, occurring more frequently. Fat drops were scattered almost in every cell. Their localization was on the whole like the one observed in the preceding stage. In one of the glandular epithelia the drops of fat were sometimes crowded together in small conglomerations. In the connective tissue close to the epithelium several fat drops were also found, in some cases even at a fair distance from the epithelial cells. Many fat drops were observable close to the small blood vessels in the interstitial connective tissue.

Preparations taken 20 days after castration. In this stage, also, fat occurred in all the examined prostatic epithelia. The drops of fat were somewhat smaller than in the preceding stage. They were present in almost every cell and were very numerous. In two of

the examined epithelia conglomerations of fat drops could be observed. In the third case the drops were more scattered.

In this stage, too, the fat drops were chiefly found in the most basal portions of the cell, but they could now more frequently be seen in increased numbers in the more apical parts of the cell. No fat drops were found in the lumina of the glandular ducts.

Numerous fat drops were now found in the connective tissue supporting the epithelium. As the plate shows they could be found at a fair distance from the epithelium. A grouping close to the blood vessels was observable in these preparations, too.

Preparations taken 60 days after castration. In this stage fat was also found in all the epithelia examined. Probably owing to the small size of the epithelial cells, the number of fat drops had decreased somewhat in this stage. The fat drops were noticeably smaller in volume than before. They were, however, very numerous and were present in most cells.

Some cells were wholly filled with fat drops. No signet-ring-resembling cells occurred. In the cells where the fat drops were few in number they could not be observed to occupy any typical position in the cell. They were scattered all over the cell. Fat drops were also present in the lumina of the glandular ducts.

Around the glandular ducts in the surrounding connective tissue fat drops often occurred at a comparatively great distance from the epithelium. In castrated rabbits well preserved fat drops were also seen sometimes in chondriome and Golgi preparations. No signs indicating a close connection between the fat drops and these cell constituents could be observed.

DISCUSSION.

Kinoshita's (1920) investigation shows that the fat content of the prostatic epithelium varies in different animal species. This investigation showed that the epithelial cells in the prostate gland of the normal full-grown rabbit does not, as a rule, contain visible lipoids. *Kunze* (1922) found lipid drops basally in the cells of the prostatic epithelium in dogs of all ages. *Policard* and *Noël* (1920) found two kinds of lipid drops in men and dogs. In the most basal portions of the cell some drops consisting of neutral fat were found. In the apical cell portions extremely small phosphatid drops occurred. The latter could also be observed in a more basal

position. According to *Kinoshita* small drops were found in the most basal portions of the cells of the prostatic epithelium, both below and lateral of the basally situated nucleus. This localization corresponds to that in normal rabbits in which lipoids could be observed in the prostatic epithelium. The presence of such fat drops did not seem to be clearly connected with any particular stage in the cycle of cellular activity. Thus we are not here concerned with metabolic products as was assumed by *Kinoshita* with regard to the occurrence of fat drops in the prostatic epithelium of normal rabbits did not appear in these investigations. These prostatic epithelia otherwise exhibited no signs of regressive changes.

After the stimulating effect of the testicular hormone on the prostatic gland has ceased, a progressive atrophy of the glandular epithelium sets in. Only a few days after the supply of hormones has ceased, the first small fat drops appear in the most basal portions of the cell. Then the quantity of lipoid gradually increases but the fat is always chiefly localized in the most basal parts of the cell where it occurs in the form of fairly small drops between the nucleus and the base of the cell and also intranuclearly. Only in stages of far advanced epithelial atrophy did the fat drops occur more irregularly scattered in the cell. The volume of the fat drops had also decreased. Signet-ring-resembling fat cells were observed on no occasion. In the basal portions of the epithelial cells of the prostate in castrated rats, *Moore, Price and Gallagher* (1930), in osmium-stained preparations, observed greenish and grey drops. They did not mention the nature of these drops, but a comparison between the observations made by these writers and similar observations on preparations made in the present investigation makes it probable that fat drops also occur in the prostatic epithelium of the rat after castration.

In the lumina of the glandular ducts fat drops were observed only after prolonged castration. In this connection it should be pointed out, that proper fat examinations were not made in the present investigation on the basis of the prostate

secretion itself. When histological preparations of the glandular tissue are prepared, most of the secretion of the gland as a rule flows away.

Small fat drops are visible in the connective tissue close to the epithelium some time after castration. They occur in particular abundance in the neighbourhood of the small blood vessels, which are found in the connective tissue. Because the fat drops first appear in the basal portions of the cells and later on, in increasing quantities, further out in the connective tissue, and on account of their abundant occurrence close to the blood vessels in the connective tissue it seems, as if the fat drops served as a transport of substance away from the epithelium and the whole organ. This might constitute a form of fatty degeneration of the prostatic epithelium.

All the fat drops observed in this investigation were singly refractive in polarized light. The same applies to the fat drops which commonly occur in the cells of the prostatic epithelium in full-grown men (Romeis 1926, Stieve 1930).

The atrophy of the epithelium under the conditions here described greatly resembles the atrophy appearing in connection with so-called hypertrophy of the prostate. — *Griffiths*, as early as 1895, observed that numerous fat drops were found in the basal portions of the cells of the prostatic epithelium in a man with a hypertrofied prostate 18 days following bilateral castration. The author's account does not make it clear whether these lipoids developed in consequence of the hypertrophy of the prostate or because of the castration.

SUMMARY.

1. In the prostatic epithelium of the normal full-grown rabbit no morphologically visible lipid drops are as a rule found.

2. In some prostatic epithelia, however, small fat drops can be observed in the most basal portions of the cell. They seem to occur independently of the cellular activity in the prostatic epithelium.

3. If the stimulating effect of the testicular hormone on the prostatic epithelium ceases, small fat drops occur first in the basal portions of the epithelial cells. Later on they also occur in increasing numbers in the surrounding connective tissue, chiefly in the vicinity of the small blood vessels of the connective tissue close to the epithelium. In stages of advanced epithelial atrophy the fat drops are again smaller and less numerous.

4. All the fat drops observed in the present investigation were singly refractive in polarized light.

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DIE VERÄNDERUNGEN IM GEFÄSSEPITHEL BEI ARTERIOSKLEROSE.

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1. EINLEITUNG.

Bei den zahlreichen Untersuchungen, die über die Anatomie, Ätiologie und Pathogenese der Arteriosklerose angestellt wurden, bildete die Gefässintima den Gegenstand eingehender Studien, indem allgemein angenommen wird, dass Intimaveränderungen bei diesem Leiden das Primäre sind, wenn auch von einzelnen (Thoma) den Mediaveränderungen Bedeutung beigelegt wird. Die bisherigen Untersuchungen beschäftigten sich besonders mit den subepithelialen Gewebsschichten, während der anderen Gewebskomponente, dem Epithel, verhältnismässig wenig Aufmerksamkeit geschenkt wurde; der vorliegenden Literatur nach zu urteilen, ist sie keiner systematischen Kontrolle unterworfen worden.

Dies rührt vermutlich daher, dass die angeblich ausgeprägtesten Veränderungen bei der Arteriosklerose in den tieferen Schichten der Intima auftreten. Ausserdem dürften feinere cytologische Veränderungen im Epithel sich mit Hilfe der üblichen Schnittmethode schwierig beobachten lassen, da man sich in der Schnittebene nur schlechte Übersicht über die einzelnen Epithelzellen verschaffen kann. Die Methode erlaubt auch keinerlei Studium der Reaktion der Zellen als Teile eines Organs, der Epithelmembran.

2. EIGENE UNTERSUCHUNGEN.

a. Fragestellung. Methodik. Material.

Die vorliegenden Untersuchungen konzentrierten sich im wesentlichen auf das Verhalten des Gefäßepithels, die tieferen Schichten der Intima wurden aber ebenfalls systematisch kontrolliert. Sowohl unter normalen als unter pathologischen Verhältnissen wurde in der Aorta das Epithel verschiedener Gebiete untersucht, jedoch unter besonderer Berücksichtigung der Stellen, wo atheromatöse und arteriosklerotische Veränderungen vorzugsweise aufzutreten pflegen, wodurch der Nachweis eventueller pathogenetischer Momente angestrebt wurde. Die Untersuchungen wurden vorgenommen an Menschen in den verschiedenen Altersklassen, um die Epithelveränderungen in den verschiedenen Stadien der Atheromatose und Arteriosklerose studieren zu können, und wo möglich feststellen zu können ob diese im Vergleich zu den Veränderungen in den tieferen Schichten der Intima primär oder sekundär sind. Falls die Ablagerung von Lipoiden, welche die beiden Prozesse begleitet, auf einer direkten Durchtränkung vom Blut aus beruht, wie von einzelnen Forschern angenommen wird (*Virchow, Aschoff, Anitschkow*), dürfte sich dieser Vorgang zu erkennen geben in Gestalt nachweisbarer Lipoidtröpfchen in oder zwischen den Epithelzellen.

Eine bislang ungelöste Frage ist ferner die Beziehung der Intimaveränderungen zu dieser Lipoidablagerung bei der Arteriosklerose und Atheromatose, indem mehrere Forscher die abgelagerten Stoffe als die unmittelbare Ursache der Intimahyperplasie auffassen, während andere annehmen, dass die bereits hyperplastische Intima diese Stoffe »abfange«.

In dem man den Zustand des Epithels in den verschiedenen Altern verfolgt, kann man sich Gelegenheit verschaffen das Verhalten desselben bei der in jugendlichem Alter auftretenden Atheromatose zu vergleichen mit dem bei der Arteriosklerose der höheren Altersklassen, und möglicherweise Momente ausfindig machen, die das gegenseitige Verhältnis dieser beiden Prozesse beleuchten, was gleichfalls noch eine

umstrittene Frage ist. So werden die beiden Prozesse von einzelnen Verfassern (*Virchow, Aschoff*) als völlig voneinander unabhängig aufgefasst, während andere die Atheromatose als das Anfangsstadium der Arteriosklerose ansehen, wodurch erstere durchaus nicht als gutartiger stationärer Prozess ohne klinische Bedeutung betrachtet werden dürfte.

Die vorliegenden Untersuchungen zielten weiterhin darauf ab, das Regenerationsvermögen des Gefässepithels in der Umgebung der arteriosklerotischen Geschwüre zu studieren, und sich an Hand der möglicherweise auffindbaren Potenzen eine Grundlage zu verschaffen, die Schlüsse bezüglich seiner Beziehung zu anderen Deckgeweben zulies und seine Einreihung unter dieselben.

Die Untersuchungen wurden vorgenommen an Menschen in den Altersklassen von 0 bis 84 Jahren. Insgesamt wurden 62 Fälle untersucht, bei denen angenommen werden durfte, dass nicht die Todesursache histologische Veränderungen bedingt, die zu eventuellen Fehlerquellen Anlass geben konnten. Die Proben wurden in sämtlichen Fällen 12 Stunden nach Eintreten des Todes entnommen, derart dass die postmortalen Veränderungen in dem gesamten Material etwa die gleichen sein dürften. Die Proben wurden an verschiedenen Stellen der Aorta entnommen, in einigen Fällen wurden ausserdem die *Art. carotis* und *femoralis* untersucht.

Die histologische Technik bestand zum wesentlichen Teil in der Herstellung von Häutchenpräparaten vom Epithel sowie von den tieferen Schichten der Intima. Die verwendete Technik wurde von mir früher angewandt und beschrieben bei Studien über die serösen Häute, die Gefässe und die Gelenksynovialis. Daneben kamen die üblichen Schnittpräparate zur Anwendung. Die Färbung der Präparate geschah routinemässig nach der Heidenhainschen Eisenhämatoxylinmethode. Ausserdem wurden verschiedene Spezialfärbungen angewandt, die im wesentlichen der Sichtbarmachung von Lipoiden, mucinöser Substanz und von elastischen Fasern dienen sollten. Zum Nachweis von Lipoiden wurde auch bei einer Reihe von Präparaten Imprägnierung mit Osmiumpräparaten gebraucht.

Zur Imprägnierung der Grenzlinien der Epithelzellen verwendete ich eine 1%ige Silbernitratlösung.

b. Anatomische Befunde.

Um eine Grundlage zu besitzen für die Beurteilung des Verhaltens des Gefäseepithels unter pathologischen Zuständen, sei hier eine kurze Übersicht gegeben über die Normalanatomie des Epithels in der menschlichen Aorta. Diese stimmt im wesentlichen überein mit dem histologischen Bild des Aortaepithels beim Kaninchen, das früher von mir beschrieben wurde.⁸⁾

Das Epithel bildet überall eine zusammenhängende Zellschicht, in der präformierte Öffnungen, Stigmata oder Stomata, nicht nachgewiesen werden können. Das Epithel in der Aorta besitzt ausgeprägt abgeflachte Spindelform (Abb. 1) mit ovalen Kernen, die gewöhnlich einen kleinen Nukleolus enthalten. In der Vena cava ist die Form der Epithelzellen dagegen deutlich polygonal, und die Kerne sind mehr abgerundet scheibenförmig (Abb. 2). Dieser Unterschied der Zellform in den Arterien und Venen ist, wie ich beim Kaninchen zeigen konnte, bedingt durch den verschiedenen Kontraktionszustand der Gefässwand. Auch bezüglich der feineren cytologischen Verhältnisse ist die Ähnlichkeit zwischen der Epithelmembran der menschlichen und der Kaninchenaorta recht gross. Nur auf einen Umstand möchte ich hinweisen, den ich bei der menschlichen Aorta stärker ausgeprägt fand, nämlich dass, dem Abgang der Intercostalarterien und anderer von der Aorta abgegebenen Äste, entsprechend, gewisse Abweichungen in der Form des Epithels vorkommen. Die Zellform ist nämlich um die abgehenden Äste herum nicht wie sonst in der Aorta länglich (Abb. 3a), sondern deutlich polygonal (Abb. 3b). Dies tritt besonders deutlich unmittelbar unterhalb der abzweigenden Arterie hervor. Die Annahme dürfte daher berechtigt sein, dass der Kontraktionszustand der Aortenwand um die Abgangsstelle dieser Arterien herum von der Norm abweicht. Die Epithelzellen in diesen Gebieten sind ausserdem stärker abgeflacht als gewöhnlich, was darauf hindeuten

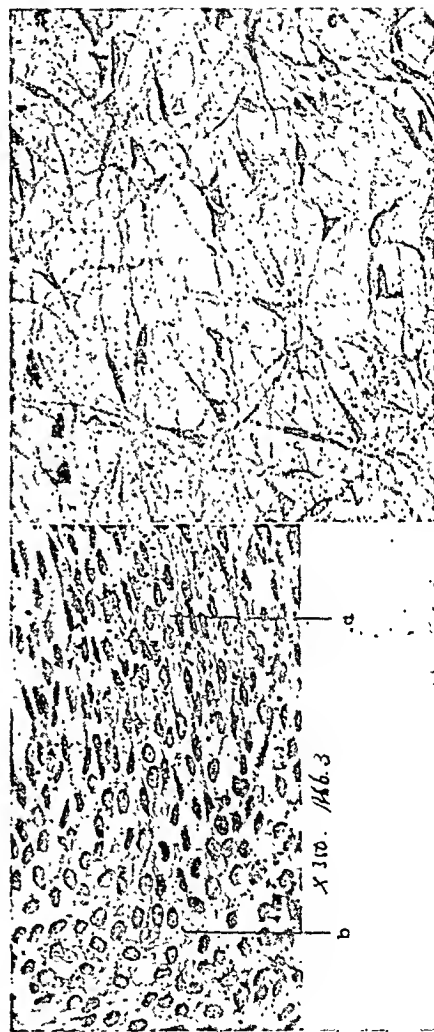


X 1000

Abb. 1

X 1000

Abb. 2



X 310. 146.3

b

a

X 400

Abb. 4

könnte, dass die Distensionskräfte in diesen Gebieten stärker sind als in der übrigen Gefässwand, wenn man dies im Zusammenhang sieht mit den elastischen Kräften der Gefässwand. Das Epithel der Gefässwand kann mit anderen Worten hier einer stärkeren Dehnung ausgesetzt sein als an anderen Stellen; dasselbe gilt auch von den elastischen und muskulären Elementen der Gefässwand. Auch in diesen Partien konnte ich niemals Öffnungen zwischen den Epithelzellen nachweisen.

An den genannten Stellen findet man ausserdem, sogar in der Aorta bei Neugeborenen, nicht selten Epithelzellen, die sich in Degeneration befinden, auch findet man in der Regel mehr zweikernige Zellen als gewöhnlich. Dies lässt sich in dem Sinne deuten, dass die physiologische Abnutzung in den Gebieten, wo Äste abgegeben werden, am stärksten ist, und dass auch möglicherweise die Regeneration hier am lebhaftesten ist. Ob diese abweichende Anatomie des Epithels in irgendeiner Verbindung steht zu dem Umstand, dass Atheromatose und Arteriosklerose eben dieselben Gebiete bevorzugen, darauf soll weiter unten eingegangen werden.

Das Epithel sowohl in der Aorta wie auch in der Hohlvene zeigen im übrigen langsames physiologisches Absterben, indem Zellen mit degenerativen Veränderungen selten zur Beobachtung kommen. Als Folge davon sieht man bei Erwachsenen nur ausnahmweise Zeichen von proliferativen Vorgängen im Epithel. Dasselbe gilt auch von den jugendlicheren Altersklassen, und selbst bei Neugeborenen sind Epithelzellen in Mitose ein ausnahmsweiser Befund.

Bei den frühen Formen der Atheromatose, die im vorliegenden Material in der Altersgruppe 10—15 Jahre beobachtet wurden, von anderen Verfassern (Zinzerling, 1925) bereits im frühen Kindesalter nachgewiesen wurden, findet man, dass das Epithel frühzeitig Veränderungen aufweist. Die Zellen nehmen ausgesprochen polygonale Form an, ohne dass ich jedoch finden konnte, dass ihr Höhendurchmesser zunahm, was von einigen Verfassern angegeben wurde, vielmehr wird ihre Plattenform noch stärker hervortretend. Die Kerne neh-

men deutlich die Gestalt runder Scheiben an, und das ganze Aussehen erinnert an normales Venenepithel. Deutlich degenerative Veränderungen der Kerne oder des Cytoplasmas werden über den kleinsten atheromatösen Herden nicht vorgefunden. Auch intrazelluläre Vakuolen konnte ich nicht nachweisen, die auf eine Ablagerung von lipoider Substanz in den Zellen hindeuten konnte.

In dem subepithelialen Gewebe der Intima findet man in diesem Stadium bereits recht prägnante Veränderungen. Das normalerweise vorhandene zelluläre Netzwerk (Abb. 4) verschwindet, und man sieht wie die Zellkerne alle Stadien von beginnender Pyknose bis zur völligen Degeneration aufweisen. Die cytoplasmatischen Ausläufer zeigen anfangs leichte Schwellung, und werden weniger deutlich als sonst gefärbt. Schliesslich werden sie ganz undeutlich und lassen sich dann nicht mehr mit Sicherheit von der strukturlosen, metachromatischen Interzellulärsubstanz unterscheiden (Abb. 5). Man erhält auf diese Weise ziemlich grosse zellfreie Partien ohne sichere Strukturierung, die von Mucikarmin rötlich gefärbt werden, und daher vermutlich schleimartiger Natur sind. In dem diese Partien umgebenden Gewebe kommt es oftmals zu einer Zellinfiltration, allerdings mässigen Grades, deren Zellbestand im wesentlichen aus Lymphocyten besteht, dagegen nur aus spärlichen Mengen grösserer mononukleärer Zellen, die keine sicheren Anzeichen phagocytärer Wirksamkeit aufweisen. Überzeugende Zeichen einer Fettablagerung trifft man bei diesen rein lokalen degenerativen Veränderungen in der Intima nicht an, und solche Veränderungen nimmt man daher in scheinbar völlig normalen Gefässen bei ganz jungen Menschen wahr. Infolge ihres anatomischen Aussehens dürften sie aber trotzdem zu frühen Stadien der Atheromatose in Beziehung zu setzen sein.

Hand in Hand mit der Grössenzunahme der atheromatösen Flecken in der Aortenwand treten deutlichere Veränderungen im Epithel auf in Gestalt einer recht erheblichen Zellpolymorphie und sicherer degenerativer Veränderungen in den Zellen. Diese Veränderungen geben sich daran zu erkennen,

dass die feineren Cytoplasmaformationen, Mitochondrien und Golgiapparat, sich nicht darstellen lassen, sowie daran, dass pyknotische Veränderungen in den Zellkernen auftreten. Die Kerne können in vielen Fällen gross und aufgebläht sein, wobei die Grösse nicht selten ein Mehrfaches der gewöhnlichen ist, zu Anfang können sie jedoch angenähert normale Form besitzen. Später treten intra- und perinukleäre Vakuolen auf, wodurch der Kern deformiert wird, und meisten Nieren- oder Hufeisenform annimmt (Abb. 6). Dies kann bei der weiteren Degeneration zu einer Abschnürung des Kerns in mehrere Bruchstücke Anlass geben. Diese Erscheinung darf aber nicht mit einer Zellteilung oder anderen proliferativen Vorgängen verwechselt werden, da es sich um einen rein degenerativen Prozess handelt.

Auch nicht während dieses Stadiums findet man Zeichen einer intra- oder extrazellulären Ablagerung von fettartigen Stoffen, dagegen zeigt das subepitheliale Gewebe reichliche Ablagerung derartigen Materials. Der überwiegende Teil dieses Materials wird frei im Gewebe liegend vorgefunden, dagegen nur eine geringe Menge in phagocytierenden Zellen. Den atheromatösen Partien entsprechend findet man nun in der Intima beträchtliche degenerative Veränderungen mit völliger Zerstörung des subepithelialen Gewebes und erheblichen Veränderungen der Membrana elastica. Ein nennenswertes Aus-treten von Fremdzellen wird dagegen nicht beobachtet.

Die hier beschriebenen Intimaveränderungen, die bei den ausgebreiteteren Formen der Atheromatose zur Beobachtung kommen, und in meinem Material oft in den jugendlichen Altersklassen auftreten, gehen ohne deutliche Grenze über in die histologischen Veränderungen, die bei der regelrechten Arteriosklerose angetroffen werden. Auch die ulzerierende Form der Arteriosklerose weist keine grundsätzlichen Abweichungen von dem geschilderten Bild auf. Dort, wo Geschwüre im Begriff stehen sich zu bilden, findet man im Epithel irreversible Veränderungen des Schluss-Stadiums der Zellablösung. Ausserdem sieht man ziemlich viele epitheliale Riesenzellen, deren Kernzahl starkt wechselt (Abb. 7); so sieht man

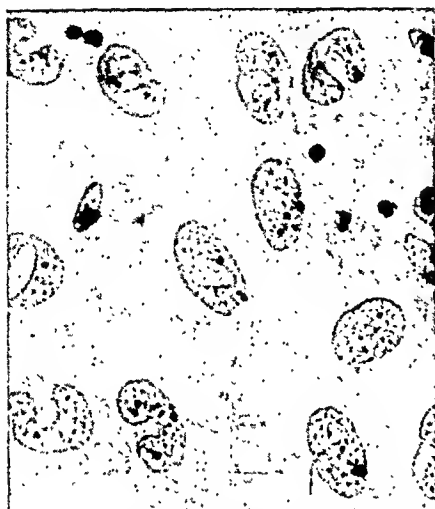


Abb. 6

× 600

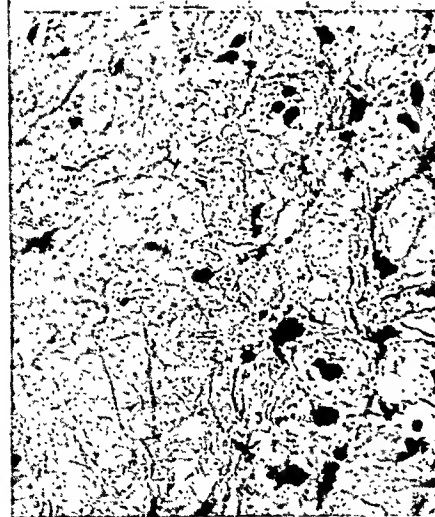


Abb. 5

× 400



Abb. 8

× 1000



Abb. 7

× 1000

zum Beispiel nicht selten Zellen, die mehr als 20 Kerne enthalten. Diese Zellen können anfänglich distinkte Grenzen besitzen, die sich daher auch mit Silbersalzen imprägnieren lassen (Abb. 7). Falls die Nachbarzellen sich abgelöst haben, werden die Cytoplasmagrenzen undeutlich, auch färbt sich das Cytoplasma mehr basophil. Einschlüsse von fremder Substanz konnte ich nicht wahrnehmen im Cytoplasma dieser Zellen. Die Zellkerne sind gewöhnlich etwas kleiner als im gewöhnlichen Gefässepithel, und die Kernform ist mehr ovoid. Oft weisen die Kerne Zeichen der Degeneration in Gestalt von Pyknose und unregelmässiger Konturierung der Kernmembran auf; ein deutliches Cytozentrum ist nicht vorhanden, ebensowenig Centriolen. Die Kerne liegen häufig zentral im Cytoplasma ohne bestimmte Anordnung; nicht selten werden sie mehr peripher angetroffen, kreis- oder hufeisenförmig angeordnet. Ebenso wie die Kernzahl schwankt auch die Menge des Cytoplasmas stark, was den Zellen ein stark polymorphes Aussehen verleiht. Diese Zellen besitzen täuschende Ähnlichkeit mit den Riesenzellen, die von mir früher beschrieben wurden im Gefässepithel des Menschen nach Röntgenbestrahlung mit grossen Dosen und harten Strahlen. Ihre Genese dürfte auch an Hand dieser Untersuchungen vermutlich in derselben Weise zu erklären sein, nämlich als das Produkt einer üblichen Kernmitose unter Ausbleiben der Cytoplasmaabschnürung im Diasterstadium.

Ist es zur Ausbildung regelrechter Geschwüre gekommen, findet man zahlreiche Riesenzellen in dem umgebenden Epithel. Auf Grund ihrer Lokalisierung an Stellen, an denen Epithelzellen in grosser Zahl zugrundegehen, und auf Grund ihrer Morphologie und Genese, liegt es nahe sie als proliferatives Produkt der normalen Epithelzelle aufzufassen, die infolge des pathologischen Wachstumsmilieus ein eigentümliches Aussehen erhält. In der Umgebung der Defekte findet man übrigens ebenfalls ein wechselndes Zellbild. Man findet zum Beispiel ganz kleine Epithelzellen (Abb. 8) mit spärlichem Cytoplasma und Kernen, die etwa halb so gross sind wie die normalen. Von diesen Zellen sind einige deutlich degeneriert

und können als stark geschrumpfte normale Zellen gedeutet werden, man findet aber auch solche kleinen Zellen, die keine histologischen Zeichen einer Degeneration aufweisen. Diese Zellen sind ausnahmslos einkernig, und weisen überhaupt keine Zeichen einer proliferativen Wirksamkeit auf. Um die Ulzerationen herum können in dem umgebenden Epithelsaum Zellen angetroffen werden, die sich in gutem Kontakt mit ihren Nachbarzellen befinden, und daher leicht imprägnierbare Grenzlinien besitzen. Dies deutet darauf hin, dass der destruktive Prozess sowie die Ausbreitung des destruktiven Prozesses einen äusserst chronischen Verlauf besitzen.

Zwischen den Epithelzellen findet man verhältnismässig wenige ausgetretene Zellen, dagegen kann man dem Wundboden entsprechend einen reichlichen fibrinösen Belag finden, der von Leukocyten infiltriert ist, und der wahrscheinlich bedingt ist durch direkte Abscheidung aus dem strömenden Blut. Regelrechte Thrombenbildung wurde dagegen selten nachgewiesen in dem vorliegenden Material.

In dem subepithelialen Gewebe findet man in diesem ulzerativen Stadium ebenfalls im wesentlichen regressive Veränderungen, während die reparatorischen Prozesse völlig in den Hintergrund treten. Immer wird dagegen eine deutliche Intimaverdickung vorgefunden, die jedoch eher durch abgelagerte Substanz bedingt ist als durch aktive Proliferation von seiten des Gewebes. Die Entzündungszellenreaktion ist verhältnismässig gering, allerdings findet man einige Lymphocyten und einige wenige phagoeytierende Zellen. Zeichen einer nennenswerten Reaktion von seiten der Vasa vasorum konnte ich nicht feststellen.

3. ÜBERSICHT.

Durch die klassischen Untersuchungen von *Anitschkow* über experimentelle Cholesteatose sowie spätere Arbeiten von *Anitschkow* und seiner Schule (*Chalatow*, 1914, *Wolkoff*, 1924, *Zinzerling*, 1923) und durch den Nachweis der Ähnlichkeit dieses Prozesses mit der menschlichen Atheromatose

und Arteriosklerose wurde die Pathogenese der letzteren in ein neues Licht gerückt. Bei der menschlichen Atheromatose findet man gleichfalls, dass der überwiegende Teil der abgelagerten Lipide aus Cholesterin besteht (*Windaus*, 1910), ein kleiner Teil dagegen aus Phosphatiden (*Schönheimer*, 1926) og Galaktosiden (*Kimmelstiel*). *Aschoff* vertrat hier die Ansicht, die schon von *Virchow* angedeutet worden war, dass diese Ablagerung geschehe durch direkte Durchtränkung der Gefässwand mit lipoidhaltigem Plasma, und die gleiche Genese wird von *Anitschkow* angenommen bei der experimentellen Cholesterinablagerung.

Indem ich Epithel von verschiedenen Stellen der normalen menschlichen Aorta untersuchte, konnte ich eine Abweichung der Form des Epithels um die Abgangsstellen der Intercostalarterien herum, besonders in der Partie unterhalb derselben, nachweisen. Die Kontinuität der Membran ist aber in keiner Weise unterbrochen, und es gelang mir nicht bei meinen Versuchen an menschlichem und tierischem Material Anhaltspunkte dafür zu gewinnen, dass das Epithel an diesen Stellen Permeabilitätsverhältnisse aufweist, die es in eine Sonderklasse stellen. Auch die Ablagerungsverhältnisse und die übrigen funktionellen Eigenschaften bieten keine sicheren Unterschiede dar. Die etwas eigenartige Morphologie dieser Stellen ist vermutlich in Verbindung zu setzen einerseits mit den abweichenden Spannungsverhältnissen in der Gefässwand, die bedingt sind durch die veränderte Architektur der elastischen und muskulären Elemente, welche die Abgabe von Ästen mit sich führt, andererseits mit Veränderungen des intravaskulären Drucks und der Strömungsverhältnisse im Blut. Die nachgewiesenen normalanatomischen Epithelveränderungen können daher schwerlich als Erklärung dafür dienen, dass die genannten Stellen bei der normalen und experimentellen Atheromatose Prädilektionsstellen für die Ablagerung von Lipiden (*Anitschkow*) sowie für die Ablagerung eingeführter kolloidaler Farbstoffe bei Tieren sind (*Glasunow*, 1926, *Okuneff*, 1926).

Durch Untersuchung des Epithels in der Aorta in ver-

schiedenen Altersklassen konnte ich nachweisen, dass man bei der beginnenden Atheromatose die frühzeitigsten Veränderungen im subepithelialen Gewebe der Intima vorfindet, und erst später im Epithel. Bei der experimentellen Cholesterinsteatose bei Tieren wird von verschiedenen Untersuchern angegeben (*Wacker u. Hueck, 1913, Saltykow, 1914, McMeans, 1915, McMeans u. Klotz, 1916, Bailey, 1916, Stuckey, 1912, Steinbiss, 1913*), dass man in der Regel intraepitheliale Lipodtröpfchen wahrnimmt, bevor eine Ablagerung von solchen in den tieferen Schichten der Intima nachgewiesen werden kann. Bei Verwendung der gewöhnlichen Schnittmethode, welcher sich die genannten Verfasser bei ihren histologischen Untersuchungen bedienen, ist es aber erstens nicht immer leicht selbst in der normalen Kaninchenaorta das Epithel zu Gesicht zu bekommen, und zweitens kann unter diesen experimentell-pathologischen Bedingungen das Gefäßepithel leicht mit Makrophagen verwechselt werden, die an der Innenseite der Gefäße angetroffen werden und fettartige Stoffe enthalten können.

Selbst dort, wo in den tieferen Schichten der Intima reichliche Ablagerung von Lipoiden stattgefunden hatte, konnte ich niemals intra- oder interepitheliale Fettröpfchen nachweisen. Dagegen konnte ich in den tiefen Schichten der Intima, ehe es zu einer nachweisbaren Ablagerung von Lipoid kam, lokale Partien feststellen können, die deutliche mucinöse Degeneration von Bindegewebe und Grundsubstanz aufwiesen, unter Auflösung des normalen fibrillären Netzwerks. Der von mehreren Verfassern vertretene Standpunkt, dass die Lipoidablagerungen das Primäre und die degenerativen und hyperplastischen Veränderungen in der Gefäßwand sekundär seien, erhält durch das vorliegende Material keine Stütze. Dieser Standpunkt stimmt auch nicht mit dem überein, was man bei der Fettdegeneration in anderen Organen findet.

In den Frühstadien des atheromatösen Prozesses findet man in der Intima keine leukocytaire Zellreaktion, weder zwischen den Epithelzellen noch in den tieferen Schichten, und das abgelagerte lipoiden Material liegt grossenteils extrazellu-

lär. Die primäre Degeneration in den tieferen Schichten der Intima gibt Anlass zu proliferativen Prozessen im Bindegewebe, und diese Intimaproliferation ist deshalb nachweisbar bevor es zur Ablagerung von lipoider Substanz kommt. Ob diese Proliferation durch die Ablagerung noch weiter angeregt wird, dafür liefern meine Untersuchungen keine adäquate Antwort. Gegebenenfalls müsste diese Frage zum Gegenstand einer experimentellen Untersuchung gemacht werden, wobei die betreffenden Stoffe direkt in die Gefässwand einzuspritzen wären.

Meine histologischen Untersuchungen über die Veränderungen des Gefässepithels zeigen keinen grundsätzlichen Unterschied zwischen den Befunden bei der juvenilen Atheromatose und bei den ernsten Formen der Arteriosklerose in den höheren Altersklassen, bei denen Geschwürbildung auftritt. Histologisch ist vielmehr der Übergang zwischen diesen beiden Zuständen fließend. Dies steht auch in gutem Einklang mit meiner Auffassung, dass die Epithelveränderungen sekundär sind im Vergleich zu den Veränderungen in den tieferen Teilen der Intima. Die vorliegenden Untersuchungen liefern daher keinen entscheidenden Beitrag zur Beleuchtung der Beziehung zwischen diesen beiden Prozessen, sie scheinen jedoch darauf hinzudeuten, dass ein Wesensunterschied zwischen den beiden kaum vorliegen dürfte. Dass die im jugendlichen Alter auftretende Atheromatose oft ein reversibler Prozess sein könnte, wie einzelne Untersucher behaupten, ist kaum anzunehmen auf Grund der nachgewiesenen primären degenerativen Veränderungen in den tieferen Schichten der Intima und auf Grund des mangelhaften Reparationsvermögens, welches die Gefässintima, meinen Untersuchungen zufolge, aufweist. Dass diese Form der Atheromatose nahezu stationär oder nur sehr langsam progredierend sein kann, ist dagegen mit meinen anatomischen Befunden gut vereinbar.

Das Regenerationsvermögen des Gefässepithels, den atheromatösen Flecken und den arteriosklerotischen Geschwüren entsprechend, ist sehr gering. Die nachgewiesene Hypertrophie einzelner Zellen, sowie die Bildung von Riesenzellen dürften

indessen als proliferative Tendenz aufzufassen sein. Diese geringe regenerative Fähigkeit steht auch im Einklang mit meinen früheren Untersuchungen über Wundheilung im Gefäßepithel, die ebenfalls langsam war im Vergleich zu ähnlichen Vorgängen in anderem Deckgewebe. Auch die tiefen Teile der Intima weisen ein verhältnismässig geringes Regenerationsvermögen auf. Dieses Verhalten dürfte auf die eigentümlichen Ernährungsverhältnisse dieses Organs zurückzuführen sein, denn es dürfte geradezu als avaskuläres Organ aufgefasst werden dürfen. Dies hat wiederum zur Folge, dass es unter pathologischen Verhältnissen zu einer schwachen Reaktion in Form eines Austretens von Zellen aus dem Blut kommt, welchem Vorgang wesentliche Bedeutung für manche biologischen Vorgänge beigemessen wurde (*Kjær, Carrel*). *Robertson* (1929) hat bei seinen Untersuchungen über die Aorta festgestellt, dass die beste Gefäßversorgung vorgefunden wird in der Umgebung der Abgangstellen der kleinen Arterienäste, also an den Stellen, die von den obengenannten pathologischen Prozessen bevorzugt werden. Aber, selbst wenn dies richtig sein sollte, dürften diese Gefäße kaum von Wichtigkeit sein für die Ernährung der Intima, sondern lediglich Bedeutung besitzen für die übrigen Schichten der Gefäßwand.

Die vorliegenden Untersuchungen geben zu Zweifel über die Richtigkeit der Theorie Anlass, dass die primäre Cholesterindurchtränkung der Gefäßwand den wesentlichen genetischen Faktor bei der Atheromatose und Arteriosklerose darstelle. Im gleichen Sinne sind auch tierexperimentelle Untersuchungen zu deuten über das Verhalten der Aortenwand gegenüber feinkolloidalen (Trypanblau) und grobkolloidalen Stoffen (Thorotrast).

Meiner Ansicht nach sind Atheromatose und Arteriosklerose bei im übrigen normalen Menschen anatomisch in eine Klasse zu setzen. Das Auftreten dieser Zustände dürfte jedenfalls teilweise aufzufassen sein als »Ermüdungssymptom«, als physiologische Abnutzung der tieferen Schichten der Gefäßintima, die sich besonders an Stellen zu erkennen gibt wo Disharmonie auftritt zwischen den intravaskulären Druck-

und Spannungsverhältnissen und der Widerstandskraft der Gefäßwand.

4. SCHLUSSFOLGERUNGEN.

1. Das normale Gefäßepithel zeigt an den Prädilektionsstellen der Atheromatose und Arteriosklerose morphologische Abweichungen, die als beruhend auf abweichenden Kontraktionsverhältnissen der Gefäßwand an diesen Stellen aufgefasst werden.
2. In der Gefäßwand bei Jugendlichen wurden örtliche degenerative Veränderungen in den tieferen Schichten der Intima nachgewiesen ohne feststellbare Lipoidablagerung.
3. Die Intimaproliferation ist nicht selten sekundär im Vergleich zu den degenerativen Veränderungen. Die abgelagerten Lipotide spielen schwerlich eine Rolle für diese weitere Wucherung.
4. Die Veränderungen im Gefäßepithel sind sekundär im Vergleich zu den nachgewiesenen tieferen Intimaveränderungen. Ablagerung von Lipoid im Epithel wurde nicht beobachtet.
5. Die Epithel- und Intimaveränderungen deuten darauf hin, dass zwischen Atheromatose und Arteriosklerose kein grundsätzlicher Unterschied besteht.
6. Das Regenerationsvermögen des Gefäßepithels ist auch unter diesen Verhältnissen sehr gering.
7. Eine wichtige Rolle spielen dabei wahrscheinlich die eigentümlichen Ernährungsverhältnisse der Gefäßintima. Letztere dürfte als gefäßloses Organ anzusehen sein, und demzufolge geringe zelluläre und proliferative Reaktion auf anormale Zustände aufweisen.

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ENDEMIC OCCURRENCE OF TYPHOID FEVER ON THE WESTERN NORWEGIAN COAST BETWEEN 1918—1939.

By *Th. M. Vogelsang.*

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Typhoid and paratyphoid B fevers have until quite recently occurred sporadically along the western coast of Norway. Speaking quite generally, the two diseases have each had their particular localizations. Although both converge towards the traffic centres, dispersions have occasionally occurred beyond these centres in different directions. A centre which until quite recently has given rise to nearly annual epidemics of typhoid fever is situated in the district of Stord among the rocky islands on the coast-line beyond Bergen. Cases of typhoid fever which have been recorded in this district from 1918 are shown in Table 1.

The Stord district (Fig. 1) has an area of about 130 square miles and was populated by 6,886 inhabitants on December 1. 1930. The district consists of the large Stord island and rocky slopes in the chain of smaller islands on the western Norwegian coast. On the Stord island two relatively densely populated places are found, namely Lervik and Sagvaag. Lervik, which is situated on the eastern side of the island, is the centre for local coast-line steamers. Only a few cases of paratyphoid B fever and no case of typhoid fever have occurred at this place. Sagvaag is situated on the south-western part

Table 1.

Reported cases of typhoid fever in the district of Stord during the years 1918—1939.

Year	Number of typhoid cases
1918	14
1919	4
1920	2
1921	0
1922	0
1923	6
1924	5
1925	8
1926	8
1927	2
1928	13
1929	6
1930	3
1931	11
1932	3
1933	0
1934	1
1935—1939	0
1918—1939	86

of the island, which faces the outward rocky islands. At this place several industrial factories are found. Sagvaag is also the export place for the nearby silex mines. Thus the place boasts of many farmers as well as industrial labourers. The four village storekeepers also handle the sale of milk. In this locality and its vicinity outbreaks of typhoid fever have occurred nearly every autumn for a number of years. In the autumn of 1928 11 cases appeared. All the patients, with the exception of one contact case, had got their milk from the same storekeeper at Sagvaag. This storekeeper J. informed us that he got his milk supply from 3 different farms in the vicinity of Sagvaag. The investigation, which was inspired by the outbreak of typhoid fever in 1928, failed to reveal the source of infection.

The storekeeper's mother was among the diseased and

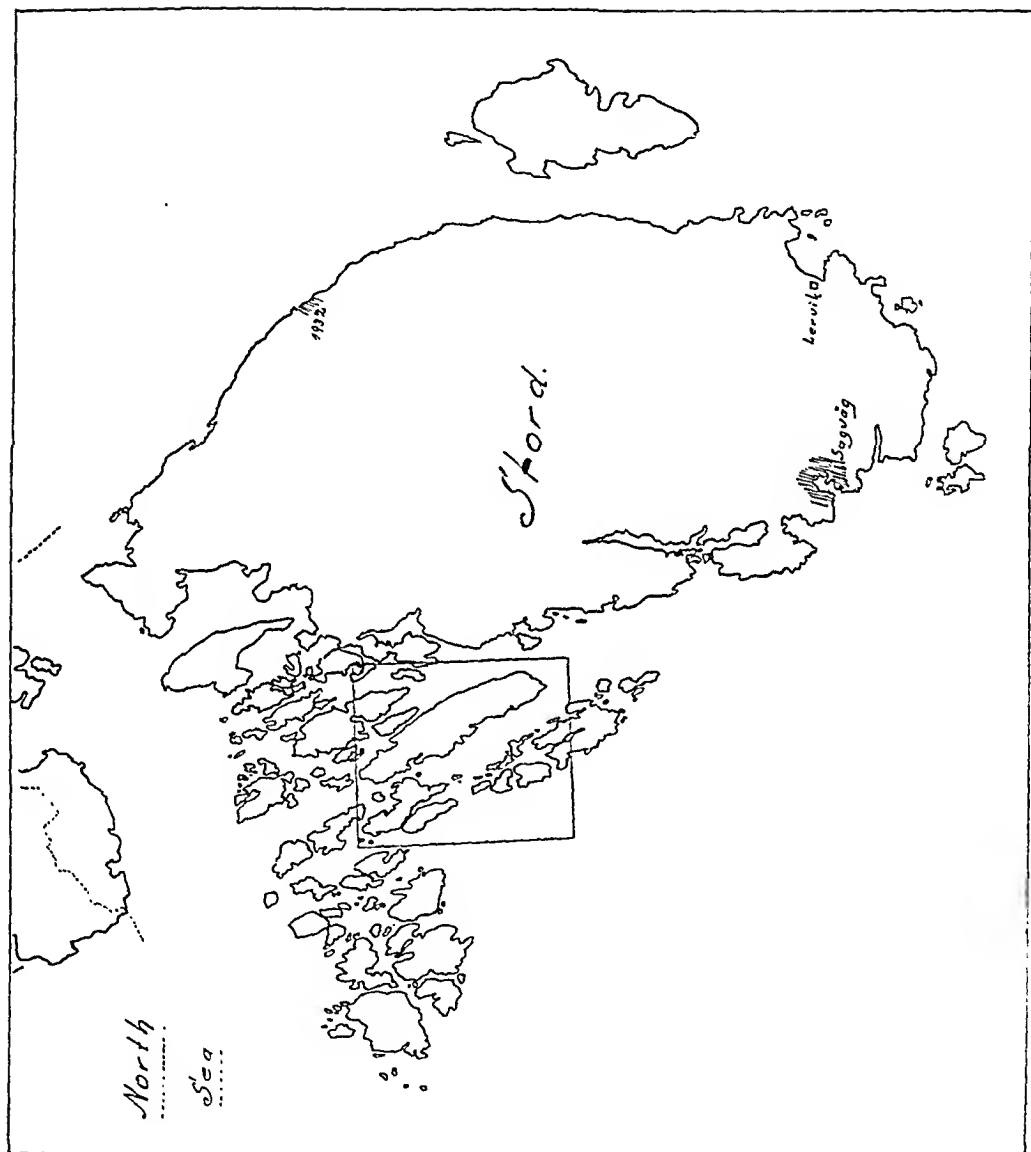


Fig. 1.

later continued to excrete typhoid bacilli with the faeces. This source of infection was suspected to be the cause of 6 new cases of typhoid fever which occurred in the neighbourhood in the autumn of 1929. However, this suspicion was quickly dispelled. This woman's husband, a former baker at Sagvaag, had been compelled to quit his business because his customers feared that he might have been infected by his carrier wife. He had now bought a house on the outskirts of Sagvaag where he and his wife lived quite isolated. After settling down at their new place, the woman-carrier had not ventured beyond the garden gate, and her storekeeper son had not seen her the last month before the outbreak of typhoid fever in the autumn of 1929.

The first two cases of typhoid fever which occurred at Sagvaag in 1929, were a labourer and his wife who took ill towards the end of September. They had never purchased the milk from storekeeper J. The last month before they took ill they got the milk from a small farm, S. The other 4 cases, however, had got the milk from storekeeper J. Meanwhile the delivery of milk to storekeeper J. had become more complicated than in 1928. When the demand for milk became unusually great, he obtained extra supplies from other farms to supplement the usual 3 ordinary supplies. Thus if one of the Sagvaag milk shops ran short of milk, they borrowed milk from another shop. Besides, every autumn during the last 5 years storekeeper J. had for some months got milk from farm S., from which the couple, which were the first persons infected in the autumn of 1928, had been supplied with milk directly.

The farm S. is situated half an hour's distance from Sagvaag. It is a rented property only large enough to maintain one cow. The house shelters the farmer and his wife and 4 small children, as well as the wife's mother, aged 75. Typhoid bacilli were isolated from this old woman's faeces. She is still alive and repeated examinations reveal that she continues to be a chronic typhoid carrier. She is the widow of a Stord farmer. When she was discovered as a typhoid carrier she

admitted that she had suffered from »a bad stomach« for several years, and had occasionally had »cramps in her stomach«. Roentgenography in 1929 revealed cholelithiasis. Except for these complaints she had never suffered from any illness. After the delivery of each of her 12 children she had been confined to bed only a few days. Several of the children had emigrated to U. S. A. No history of typhoid fever is available among her children or grand children except for a suspicious protracted fever which her son suffered from in 1913, he was treated by a quack.

After her husband's death in 1910 she continued to live on the farm until 1918 when she moved to live with a daughter not far from Sagvaag. That same year 4 cases of typhoid fever appeared in the neighbourhood, namely a labourer's wife and 3 children. No direct communication has been established between the chronic carrier and these instances of typhoid fever.

In 1920 she moved to farm S. with her daughter's family. All the cases of typhoid fever which have occurred in Sagvaag can subsequently be traced to this chronic typhoid carrier.

Thus in the summer of 1920, two brother labourers living separately in a small house near Sagvaag got their milk from farm S., by the end of July 1920 both had been attacked with typhoid fever. The house remained vacant during the next 3 years until their parents moved in in July 1923. At the same time they started getting their milk from farm S., and already in September 1923 they were both taken ill with typhoid fever. Their two sons, who had had the disease in 1920, were now employed elsewhere. Later in the year farm S. began to deliver milk to another labourer's family near Sagvaag. Subsequently, the labourer and 2 children got ill in October 1923. At the same time another labourer living a mile off was attacked with typhoid fever. A few weeks earlier this labourer had worked with electrical installation near Sagvaag and for one day had taken his meals with the aforementioned family. Among his family of 6 he was the only one who contracted the disease.

The following autumn, 1924, the family living next to the house where typhoid fever had occurred in 1920 and 1923, got their milk from S., and out of eight persons in the household five were attacked with typhoid fever. Only the three youngest children escaped the disease.

In 1925 milk was for the first time delivered from S. to the storekeeper J. at Sagvaag. This autumn typhoid fever occurred in three different places among labourers who had bought their milk from J. In one of these places the labourer himself as well as his wife were attacked. In the same house there lived three other families who got their milk elsewhere and avoided the disease.

During 1926 and 1927 no case of typhoid fever occurred at Sagvaag or its vicinity in spite of the fact that milk deliveries took place for some months in the autumn during this time both from farm S. and storekeeper J. The fact is that the chronic typhoid carrier was not staying at Stord at this time as she had left for U. S. A. in October 1925 to visit some of her children, but having attended church three times without understanding the language in which the sermon was preached, she became uneasy about ending her life under such circumstances, and she returned to her daughter at Stord in 1928.

The following autumns of 1928 and 1929 when delivery of milk from farm S. to storekeeper J.'s shop at Sagvaag had been resumed, cases of typhoid fever reappeared. In 1928 there appeared 11 cases in 10 households and 6 further cases in 5 other families in 1929. Each of the diseased persons had got their milk from storekeeper J., except the aforementioned family who received their supply directly from farm S., and a nurse who was infected in the autumn of 1928 while attending one of the other typhoid patients.

This survey reveals the fact that a total of 34 cases of typhoid fever in the vicinity of Sagvaag from 1920—1929, with the exception of one single instance of direct contact infection in 1928, can be traced to the chronic typhoid carrier

on farm S., and that the contagion has been spread by means of the milk delivered directly or indirectly from farm S.

During all these years serious consideration has been given to sundry means of contagion conveyance, such as wells and sewers, especially where the disease has frequented the same household repeatedly and has given rise to the connotation of »typhoid houses«. Thus we have seen one such household in which the disease appeared in 1920, disappeared during the vacancy of 3 years, and reappeared immediately after the arrival of a new family. Likewise in another household the disease occurred in 1923, 1928 and 1929. The farmer tenants had not become aware of the fact that each time the disease occurred in these so-called »typhoid houses«, the patients had got their milk from the same milk deliverers.

It being a milk-borne endemic one is particularly struck by the relatively high frequency of men who catch the disease. All are labourers with homes and families elsewhere who spend the entire week working at Sagvaag, partly as seasonal labourers, and who buy their milk from storekeeper J. As soon as they fall ill they return to their families and fail to call a physician until they have arrived home. The curious thing is that in spite of the late isolation of these patients, not a single case of contact infection can be pointed out in these labourers' families.

Inasmuch as all the cases of typhoid fever which have occurred in this district during the period of 1920—1929 can be explained as being caused by milk-borne infection traceable to the chronic carrier on farm S., one might expect that the disease would disappear after the source of infection had been rendered harmless by the safe isolation of the carrier. During the subsequent decade only one single case of typhoid fever occurred in this district. This case was found in 1930 and no connection could be traced with the well-known carrier. Now it is known that this case was infected from one of the small islands beyond Sagvaag where endemics of typhoid fever have been common during many years.

The distance between Sagvaag and these small islands is

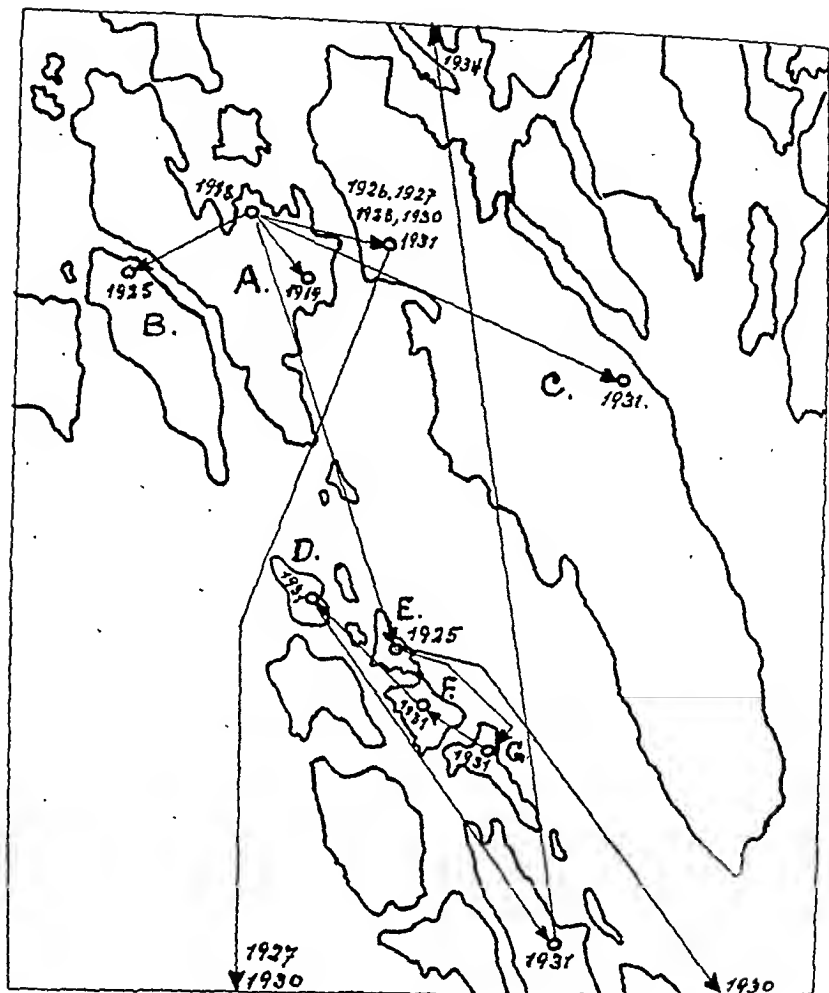


Fig. 2.

approximately 12—15 miles. These islands are situated on the outward western rocky slopes of Norway (Fig. 2). The settlements on these islands are very scattered, the communications are scarce and very little traffic goes on between the different islands. The main livelihood is fishing supplemented with very limited farming. The sale of milk takes place only rarely. The typhoid fever area is confined within about 8 square miles where sporadic cases of the disease have

occurred. The disease has spread beyond the area on a few occasions only.

In 1918 the entire household of 11 members was attacked by typhoid fever on Island A. The source of infection remains unknown. In 1931 a man on Island C became infected with the disease during a visit to Island A. Subsequent examinations revealed 2 chronic typhoid carriers on Island A, namely, 2 women aged respectively 59 and 72, the wife and the sister of the farmer. A previous single case of the disease occurred on this island in 1919 in a young girl who probably was infected by one of the two carriers already mentioned.

In 1925 a man from another district visited his parents-in-law on Island B. Immediately after his return home he was confined to bed with typhoid fever and he infected his wife and his brother. At the same time his mother-in-law and his sister-in-law were taken ill on Island B. All these infected persons were probably infected through milk delivered from the farm on Island A where the two carriers lived.

In 1931 a woman on Island G was struck with typhoid fever after a prolonged visit to Island E. Only one solitary family consisting of husband, wife and a son, lived on this latter island. Examinations revealed that the 55 years old wife was a chronic typhoid fever carrier. In 1925 she had been indisposed with diarrhoea and a severe headache for a fortnight without calling in a physician. Afterwards her husband became ill for 3 months with a malady suspected to have been pneumonia. It is probable that both were ill with typhoid fever. The family was exceedingly poor and partly supported itself by travelling about the island rag-picking. It is possible that the wife was infected during her travels on Island A or B. The following year, 1926, their only son was attacked by the disease. After convalescence these rag-pickers visited another part of the district where 3 persons were infected with typhoid fever. The aforementioned case of typhoid fever at Sagvaag in 1930 also contracted the disease after having visited this family on Island E. It is most probable, therefore, that he became infected from the carrier on this island.

On island C, which lies opposite to Island A, there is a cluster of 6 households and a store. In 5 of these households typhoid fever has occurred since 1926. In February 1926 the wife of the storekeeper fell ill with a disease regarded as pneumonia. The following month one after another got ill in this house. It was not until late in the summer that a diagnosis of typhoid fever was made. The faeces of the person first attacked was examined for typhoid bacilli with negative results. One of the storekeeper's sons married in 1926. He was among those who caught the disease that same year. In 1927 his wife and presumably also an aunt, who was staying with them, were struck with typhoid fever. Examinations carried out at this time showed that his mother, who was the first who caught the disease one year previously, was a chronic typhoid carrier. Most probably she was the source of infection of the other cases which occurred in this family.

The typhoid contagion was probably transferred from Island A to a small trading place on Island C. The storekeeper in this place was the brother of the 59 year old chronic typhoid carrier on the island. The distance between the two places is very short and the families often exchanged visits. Thus there have been adequate opportunities to contract the contagion from Island A.

The storekeeper's second son married in 1927 and continued to live with his family at his parents' home. In 1930 his wife and thereafter a neighbouring woman caught the disease. The latter died. It is most likely that both had become infected from the storekeeper's wife who had not observed the directions given her as a chronic typhoid carrier.

In 1931 this carrier's 4 year old grand-daughter contracted typhoid fever and the old carrier was again suspected to be the source of the contagion. The girl's parents were again examined inasmuch as both had had the disease in 1926—27. The patient's mother, the daughter-in-law of the old carrier, was discovered to be a chronic typhoid carrier herself. After convalescence this new carrier visited her native village in another district. Her brother caught the disease during her

stay. In 1930 a 24 year old woman from the place of trade mentioned on the island was employed as a servant in the house of the young carrier's brother. Having been there for six weeks, she returned home ill with typhoid fever and died of the disease. An extensive investigation failed to give any definitive clue about the source of infection. In view of the fact that the old carrier had sent some apples to the girl, this possibility of contagion was considered. Subsequent information revealed, however, that the younger carrier also this time had visited her brother and had shared the room with the girl.

It is probable that typhoid fever has occurred in a fifth household. Two daughters in this place protracted a febrile disease in 1928. Since the family had often been visited by the two carriers, the younger of whom had not as yet been recognized as a carrier, it is most probable that these two daughters had been infected from these carriers. The family has since then left the place.

Besides this most populated trading place on Island C, 5 other parts of the island are inhabited. The distances between these places are long and communications poor. Two or three farms are found in these places. Typhoid fever has been unknown here until 1931 when a solitary case occurred on that side of the island which lies opposite to the trading place. We have already mentioned that the patient had visited Island A. It was that case which led to the detection of the two carriers on this island.

An old hermit had been living on Island F for several years in a tumbledown muddy shanty. On the advice of a local charity organization, a medical officer visited this hermit in 1931 and found him completely emaciated and wretched. He was thereupon placed in a boarding-house on Island D, where he died a fortnight later, 80 years of age. About 50 persons attended his funeral, and were entertained at this house. Later happenings make it appear that the hermit died of typhoid fever. A solitary family, consisting of a widow, her son, her daughter, and her grand-child, lived on island G. As

mentioned above, this daughter had become infected with typhoid in 1931 during a stay on Island E. She later on continued to excrete typhoid bacilli with the faeces. The old hermit never visited anyone before he took ill except for a few trips to the Island G where he took his meals. It is probable that he caught the disease during one of these visits and carried the contagion with him to the Island D. On this island there lived a single family consisting of a married couple and their 5 children. Only a few days after the hermit's death in 1931, one of the children was confined to bed, and during the following days one after another of this entire household came down with typhoid fever.

It must be feared that a dispersion of typhoid infection might take place in the district during the time following the funeral on island D which was attended by about 50 persons. Only one single case is on record which may be traced to this event. The island H is more densely populated than the aforementioned islands. On this island a farmer aged 40 was attacked with typhoid fever a few weeks after the funeral on Island D. He was the brother of the housekeeper on Island D and had visited the island once later than the day of the funeral. Most probably he was infected during one of these visits to Island D. The disease spread from this farmer to two other persons on Island H. He was nursed during the disease by his wife who also caught the disease while their 2 sons remained well. On the other hand, the disease spread to the neighbouring farm where a 5 year old boy caught typhoid fever. In 1932 the farmer's wife was declared free from contagion after 3 negative samples of faeces and urine. In 1934 she visited her daughter who lived on an island in another part of the district. Some time after her return her son-in-law contracted typhoid fever. His mother-in-law was thereupon re-examined and was declared to be a chronic typhoid carrier. Her son-in-law is the last case of typhoid fever which has occurred on the islands scattered along the Stord coast-line.

Besides the occurrence of typhoid fever in the afore-

mentioned places, a single case occurred in 1932 in quite a different locality on Stord, where the infection of a boy was traced to his grand-mother, who proved to be a chronic carrier. She had earlier lived in another locality. Her husband had died 40 years before of »pneumonia«. At this time several cases of typhoid fever had occurred in this neighbourhood. She stated that her health had always been excellent and that so far as she knew she had never infected anybody else. She moved to live with her daughter in 1930 and at this place the aforementioned case of typhoid fever occurred in 1932.

Summary.

Bacteriological examinations carried out in the Stord district along the Western-Coast-line of Norway, have yielded a natural explanation to the occurrence of every one of the 84 cases of typhoid fever which have been recorded between 1918—1939.

A number of small endemics of typhoid fever have been milk-borne and caused by individual chronic typhoid carriers. Other instances have been spread either from unknown chronic carriers or known typhoid fever patients.

Bacteriological examinations revealed 10 chronic typhoid carriers in this district. The first carrier was detected in 1927 on Island C and caused a number of typhoid fever cases because of gross infraction in ordered precautionary measures. The remaining 9 carriers are not known to have caused any infection after their detection. Two old carriers are still living on Island A. The two oldest of the remaining carriers, respectively 85 and 88 years of age, are placed under supervision of another chronic carrier. Extirpation of the gall-bladder has been done on the four youngest carriers and three of these have already become abacillary in the faeces while the fourth continues to excrete typhoid bacilli.

DER EINFLUSS VON BAKTERIEN AUF WASSERMANN-NEGATIVE SERA. II.

Von *Olof Sievers*.

(Eingegangen bei der Redaktion am 8. Oktober 1940).

Die von *Snellman*, sowie später von *Takano* gemachten Beobachtungen hinsichtlich des Einflusses des *Bazillus subtilis* auf Wassermann-negative Sera wurden von mir im ersten Teil dieser Arbeit*) bestätigt. Dabei wurde die Bedeutung der langsam vor sich gehenden Veränderung bei diesen infizierten Sera hervorgehoben. Eine ähnlich fortlaufende Veränderung zeigte auch die nach *Takanos* Angaben infizierte Bouillon. Durch die wiederholte Untersuchung der Proben an mehreren aufeinander folgenden Tagen wurde eine Erklärung für die von einander abweichenden Ergebnisse der besagten Forscher erhalten. Sowohl *Snellman*, wie auch *Takano* führten die Reaktionen mit fixierter Komplementdosis aus 1:10, und bei meinen ersten Versuchen bediente ich mich parallel der Technik dieser beiden Forscher. Die gleiche Cereuswirkung tritt jedoch ebenso deutlich hervor, wenn die Reaktionen an Hand einer Methode ausgeführt werden, bei der die Komplementmenge variiert und jedes einzelne mal mittels Vorversuchen bestimmt wird (ad modum *Harrison-Wyler* und ad modum *Sierakowski*). Die Fähigkeit des *Bazillus cereus*, erst eine scheinbare Wassermann-Positivität mit nachfolgender Eigenhemmung hervorzurufen, ist stets, un-

*) Acta path. et microbiol. scand. 46, 365 (1939).

abhängig von den verwandten Wassermann-Modifikationen, gleich in die Augen fallend gewesen.

Ausser dieser Wiederholung früherer Versuche, jedoch mit einer anderen Wassermann-Technik, wurden in diesem Zusammenhang auch noch einige Details einer Prüfung unterworfen. So kann man sich fragen, wieweit die hier besprochenen Veränderungen auch mit Hilfe von toten Bakterien hervorgerufen werden können. Um dieses zu untersuchen wurden früher vorgenommene Versuche im einzelnen wiederholt, jedoch mit toten Bakterien an Stelle von Lebenden. Die Bakterien wurden von einer Agarkultur in 1 % iger Formalinkochsalzlösung aufgeschwemmt. Zentrifugieren nach eintätiger Aufbewahrung in 37° C, d. h. nachdem die Sterilitätskontrolle gezeigt hat, dass die Emulsion keine lebenden Bakterien enthält. Der Bodensatz wurde wiederholt mit physiologischer Kochsalzlösung gewaschen. Die so erhaltene Bakterienmasse wurde in gewöhnlicher Nährbouillon aufgeschwemmt, so dass eine Dichte entstand, die einer 24 Stunden Bouillonkultur von *Esch. coli* entsprach. Zwei Bouillonkolben diese Art (*B. cereus* bzw. *Esch. coli*) wurden bei 37° C aufbewahrt. Nach 1, 3, 7 bzw. 16 Tagen wurden diesen Kolben Proben entnommen und wie üblich einige Minuten zentrifugiert, sowie nach der folgenden Methode untersucht: 0,25 ccm dieser Bouillon (bzw. nicht infizierte Kontrollbouillon) wurden bei 37° C eine Stunde mit 0,25 ccm cholesterinisiertem Rinderherzextrakt bzw. mit physiologischer Kochsalzlösung und folgenden Mengen Komplement (Volumen 0,25 ccm) 0,025, 0,02, 0,015, 0,01, 0,008 und 0,005 ccm digeriert. Eine halbe Stunde nach dem Zusatz von 0,25 ccm Ambozeptorverdünnung (viermal die totalhämolyisierende Dosis) und 0,25 ccm 5 % ige Hammelblutkörperchenemulsion geschah die Ablesung. Die Ergebnisse zeigten, dass eine der früheren Beobachtung entsprechende Veränderung der Bouillon nicht vorkam. Trotz einer 16-tägigen Aufbewahrung zeigte die Bouillon keinen hemmenden Einfluss gegenüber der Hämolyse. Diese Ergebnisse besagen nur, dass von den mit Formalin getöteten Bakterien nichts extrahiert wurde, was die betreffenden Ver-

änderungen hervorgerufen hätte. Hiermit ist jedoch nicht gesagt, dass tote Bakterien an und für sich auf den Verlauf der Hämolyse keinen störenden Einfluss ausüben könnten (siehe z. B. *Hirszfeld* und *Klinger* sowie *Nathan*). *Takanos* Untersuchungen mit Zentrifugaten bzw. Filtraten zeigen indessen dass in der Flüssigkeit irgendetwas sein muss, das diese Veränderungen hervorrufen kann, und der Zweck dieser hier besprochenen Versuche war nur eine Prüfung, inwiefern der wirksame Bestandteil eventuell aus toten Bakterien extrahiert werden könnte. Dieser Versuch wurde mit durch Autoklavieren getöteten *Cereus*bakterien wiederholt, also ohne Verwendung von Formalin. Das Ergebnis war das gleiche, die Bouillon bekam keine hemmende Fähigkeit.

Jacobson hat durch Absorptions- und Elutionsversuche erstrebt, die durch *Subtilis* hervorgerufene Reaktion von der gewöhnlichen Wassermannreaktion zu trennen. Sie machte ihre Versuche in Übereinstimmung mit *d'Alessandro* und *Sofia* mit Kaolin und konnte keinen Unterschied zwischen natürlichen Luessera und subtilisinfierten Sera feststellen. *Sachs* ist der Ansicht, dass durch diese Versuche keine Identität der beiden Arten von Sera bewiesen wurde. Er hebt folgendes hervor: »Es wäre notwendig Bedingungen zu wählen, unter denen die natürlich wirksamen Stoffe des Luesserums eluiert werden, und dann festzustellen, ob die künstlichen Subtilisstoffe gleichfalls einer Elution zugänglich sind oder nicht«. Trotz der Berechtigung von *Sachs'* Vorschlag hinsichtlich der Fortsetzung der Untersuchungen scheint es mir begründet, auch noch näher zu prüfen, inwieweit das wirksame Prinzip direkten Einfluss auf die Blutkörperchen, das Komplement bzw. den Ambozeptor auszuüben vermag. Dieses scheint um so mehr begründet, als meine Versuche darauf hindeuten, dass die Wassermann-Positivität als ein relativ schnell vorübergehendes Stadium zu betrachten ist.

Jeder bisher vorgenommene Versuch zeigt, dass die Blutkörperchenemulsion beim Fehlen der beiden übrigen Faktoren nicht direkt beeinflusst wird. Zwar zeigt sich oft eine Farbenveränderung, indem die rote Farbe in eine Violette

übergeht, aber ein Zusammenhang zwischen diesem Phänomen und der zunehmenden Hemmungsfähigkeit scheint doch nicht zu bestehen. Je mehr Bakterien bei dem Versuch anwesend gewesen sind, um so intensiver ist gewöhnlich die Farbenveränderung hervorgetreten.

Der Einfluss des wirksamen Prinzipes auf das Komplement sei mit folgendem Versuchsprotokoll beleuchtet:

Absteigende Menge des Meerschweinchenkomplementes (Volumen 0,25 ccm) wurde $\frac{1}{2}$ Stunde mit 0,25 ccm Ambozeptor (viermal die total hämolysierende Dosis) und 0,25 ccm 5 %iger Hammelblutkörperchenemulsion, sowie mit

- I. 0,25 ccm zentrifugierter Bouillon, die zwei Tage zuvor mit *B. cereus* infiziert worden war und hernach bei 37° C gestanden hatte,
- II. 0,25 ccm zentrifugierter Bouillon, die zwei Tage zuvor mit *Esch. coli* infiziert worden war und danach bei 37° C gestanden hatte,
- III. 0,25 ccm steriler Bouillon,
- VI. 0,25 ccm physiologischer Kochsalzlösung, digeriert.

Der Versuch wurde in zwei Parallelserien gemacht, A bzw. B, wobei die Ambozeptorverdünnung und die Blutkörperchenemulsion bei der Serie B erst zugefügt wurden, nachdem die Bouillon und das Komplement zusammen 1 Stunde bei 37° C digeriert hatten. Die festgestellte Hämolyse wird in Tabelle I wiedergegeben. (Die Hämolyse wurde nach der von Madsen aufgestellten Skala angegeben).

Der erste Abschnitt der Tabelle, A, zeigt, dass die mit *B. cereus* infizierte Bouillon die Hämolyse etwas hemmte, während die übrige Bouillon diese gar nicht beeinflussten. Lässt man die zentrifugierte *B. cereus*-Bouillon eine Stunde auf das Komplement wirken, so zeigt sich, wie aus dem Abschnitt B hervorgeht, praktisch genommen gar keine Hämolyse. Es hat mit anderen Worten den Anschein, als ob das Komplement zerstört worden wäre, und als ob eine kürzere oder längere Zeit nötig gewesen wäre, damit dieses vollständig geschehen konnte. Jedenfalls hat man mit einer,

Tabelle 1.

Fallende Mengen des Komplementes in ccm	Hämolyse von Hammelblutkörperchen mittels Meerschweinchen-Komplement und Ambozeptor nach dem Zusatz von:			
	Mit <i>Cereus</i> infizierter Bouillon	Mit <i>Coli</i> infizierter Bouillon	Steriler Bouillon	Physiologi- scher Koch salzlösung
Volumen 0,25 ccm				
		A		
1/ 0,025	100	100	100	100
2/ 0,020	100	100	100	100
3/ 0,015	100	100	100	100
4/ 0,010	10	100	100	100
5/ 0,008	0	100	100	100
6/ 0,006	0	100	100	100
7/ 0,004	0	90	90	80
8/ 0,002	0	20	20	10
9/ -	0	0	0	0
		B		
1/ 0,025	10	100	100	100
2/ 0,020	0	100	100	100
3/ 0,015	0	100	100	100
4/ 0,010	0	100	100	100
5/ 0,008	0	80	90	90
6/ 0,006	0	30	40	40
7/ 0,004	0	0	0	0
8/ 0,002	0	0	0	0

wenngleich nicht vollständigen, momentanen Zerstörung zu rechnen.

Dieser Versuch wurde mit Bouillonkulturen vorgenommen, aber das gleiche, wenngleich nicht ganz so prägnante Ergebnis erhält man, wenn man zu dem Komplement lebende *Cereus*-Bakterien mischt. Auch hierbei kann man eine momentan zerstörende Wirkung verzeichnen im Gegensatz zu *Esch. coli*. Lässt man die beiden besprochenen Bakterienarten 24 Stunden das Komplement beeinflussen, so wird dieses zerstört, so dass eine Hämolyse nicht eintritt. Das Frappierende der hier erörterten Versuche ist die Geschwindigkeit der Wir-

kung der *Cereus*-Bakterien, beziehungsweise der *Cereus*-bouillon im Vergleich zu dem Einfluss der *Coli*-Bakterien.

Die Fähigkeit des *Bazillus cereus*, schnellen Einfluss auf das Komplement auszuüben, bringt einen auf den Gedanken, dass es sich hier eventuell um eine Zerstörung des dritten Komponenten des Komplementes handelt, wie es zum Beispiel die Hefezellen machen. Ein erster Versuch in dieser Richtung zeigte, dass ein durch *Cereus* zerstörtes Komplement mit in Wärme inaktiviertem Komplement reaktiviert werden konnte. In diesem Zusammenhang sei nur ein Protokoll wiedergegeben, und dieses beleuchtet die Fähigkeit der *Cereus*-Bakterien, den dritten bzw. vierten Komponenten des Komplementes zu beeinflussen:

Frisches Meerschweinchenserum wird in vier Portionen aufgeteilt und diese werden behandelt mit

a) einer 24 Stunden alten Kultur von Bäckerhefe (nach *Toda* und *Mitsuse*). Die Hefezellen wurden wiederholt mit physiologischer Kochsalzlösung gewaschen. Von dem Bodensatz wurde eine 20 %ige Emulsion in Kochsalzlösung gemacht. 0,5 ccm dieser Emulsion wurden mit 2 ccm Meerschweinchenserum gemischt. 20 min in 37° C mit darauffolgendem Zentrifugieren.

b) einer 24 Stunden alten *Cereus*-Kultur. Die Versuchsanordnung die gleiche wie bei den Hefezellen.

c) Ammoniak (nach *Gordon*, *Whitehead* und *Wormall*). 1 ccm frisches Meerschweinchenserum wurde mit 0,25 ccm n/6,5 Ammoniaklösung versetzt. Nach 1¼ stündigem Stehen bei 37° C wurde die Lösung mit 0,25 ccm n/6,5 Salzsäure neutralisiert und auf 21 ccm Volumen mit physiologischer Kochsalzlösung verdünnt.

d) Ammoniumsulfat (nach *Tokunaga*). 1 ccm frisches Meerschweinchenserum wurde 24 Stunden bei Zimmertemperatur belassen zusammen mit 0,008 gr Ammoniumsulfat.

Von diesen in obengenannter Weise inaktivierten Sera wurden 0,25 ccm vermengt mit 0,25 ccm

- 1) physiologischer Kochsalzlösung.
- 2) in Wärme inaktiviertem Meerschweinchenserum (10', 56°) 1:10 verdünnt.
- 3) mit Hefe behandeltem Serum, das danach in Wärme inaktiviert wurde (10', 56°).
- 4) mit *Cereus* behandeltem Serum, das danach in Wärme inaktiviert wurde (10', 56°).

- 5) mit Ammoniak behandeltem Serum, das danach in Wärme inaktiviert wurde (10', 56°).
 6) mit Ammoniumsulfat behandeltem Serum, das danach in Wärme inaktiviert wurde (10', 56°).

Nach dem Zusatz von sensibilisierten Hammelblutkörperchen wurden die Röhrchen 2 Stunden in ein Wasserbad von 37° C gestellt. Die Ergebnisse (die kontrolliert wurden, nachdem die Röhrchen weiterhin über Nacht im Eisschrank gestanden hatten) gehen aus der Tabelle II hervor (+ = vollständige, — = keine Hämolyse).

Tabelle II.

0,25 ccm Meer- schweinchense- rum, behandelt mit	Zusatz von 0,25 ccm Meerschweinchen- serum behandelt mit	Hä- mo- lyse
Hefezellen	physiologischer Kochsalzlösung	—
	Inaktivierung 10' 56°	+
	» » » und Cereusbakterien	—
	» » » » Ammoniak	+
	» » » » Ammoniumsulfat	+
Cereusbakterien	physiologische Kochsalzlösung	—
	Inaktivierung 10' 56°	+
	» » » » und Hefezellen	—
	» » » » Ammoniak	+
	» » » » Ammoniumsulfat	+
Ammoniak	physiologische Kochsalzlösung	—
	Inaktivierung 10' 56°	+
	» » » » und Hefezellen	+
	» » » » Cereusbakterien	+
	» » » » Ammoniumsulfat	—
Ammoniumsulfat	physiologische Kochsalzlösung	—
	Inaktivierung 10' 56°	+
	» » » » und Hefezellen	+
	» » » » Cereusbakterien	+
	» » » » Ammoniak	—

Diese Ergebnisse lassen darauf schliessen, dass die Cereusbakterien den gleichen Einfluss auf das Komplement aus-

üben wie die Hefezellen, d. h. den dritten Komponenten beeinflussen. Sowohl das in Wärme inaktivierte, wie das mit Ammoniak bzw. mit Ammoniumsulfat behandelte Komplement besass die Fähigkeit, nicht nur das mit Hefe, sondern auch in gleicher Weise das mit *Cereus* behandelte Komplement zu reaktivieren.

Nach dieser Erörterung der Zerstörung des Komplementes durch *Cereus* steht noch zu berichten, dass fortgesetzte Versuche gezeigt haben, dass die die Hämolyse hemmende Eigenschaft den Ambozeptor nicht beeinflusst. Aus der Wiederholung der hier angeführten Versuche, jedoch in entgegengesetzter Richtung vorgenommen, so dass man also die Bakterien auf Ambozeptorserum wirken liess und nicht auf das Komplement, ging hervor, dass der Ambozeptor inaktiv blieb.

Die jetzt vorgenommenen Versuche haben gezeigt, dass der *Bazillus cereus* die Fähigkeit besitzt, den dritten Komponenten des Komplementes zu beeinflussen. Die besagte Wirkung tritt nicht nur beim Zusatz von Bakterien zu Komplementserum auf, sondern auch, wenn man zu Serum filtrierte bzw. zentrifugierte *Cereus*bouillon fügt. Speziell bei den Bouillonversuchen kann man eine äusserst schnell eintretende Veränderung des Komplementes beobachten. Man fragt sich, inwiefern diese Beobachtungen eventuell von Bedeutung sind bei dem Versuch einer Erklärung der von mir hier berührten Beobachtungen von *Snellman* und *Takano*.

Mit Hinblick darauf, dass die beobachtete Wassermann-Positivität am ehesten als eine Phase in einem fortlaufenden Prozess zu betrachten ist, scheint es mir berechtigt, der Fähigkeit des wirksamen Prinzips das Komplement schnell zu beeinflussen Beachtung zu schenken. Es kann allem Anschein nach nicht ganz unmotiviert sein, hierin einen mitwirkenden Faktor des von *Takano* beschriebenen Phänomenes zu sehen.

Zusammenfassung.

1) Die Fähigkeit der Cereusbakterien, ein ursprünglich Wassermann-negatives Serum anfangs in ein Wassermann-positives, später in ein eigenhemmendes zu verwandeln, tritt unabhängig von der verwandten WR-Modifikation hervor.

2) Eine Beimischung von entweder mit Formalin oder durch Wärme (Autoklavierung) getöteten Cereusbakterien zu Bouillon kann diese nicht in eine »WR-positive« oder eigenhemmende verwandeln, wie es der Fall ist, wenn die Bouillon mit lebenden Bakterien beimpft wird.

3) Das wirksame Prinzip beeinflusst weder den Ambozeptor, noch die Blutkörperchen, während hingegen frisches Meerschweinchenserum sehr empfindlich gegenüber dem Einfluss der Bakterien zu sein scheint. Wiederholte Versuche deuten darauf hin, dass es der dritte Komponent des Komplementes wäre der, hierbei beeinflusst wird. Die Ergebnisse sprechen für eine Übereinstimmung der Wirkungsart zwischen Cereusbakterien und Hefezellen.

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MAKROSKOPISCHE UND MIKROSKOPISCHE UNTERSUCHUNGEN DER SCHLEIMHAUT DES KANINCHENMAGENS IN IHRER REAKTION AUF RÖNTGENSTRAHLEN.

Von *Erling A. Mylius*.

(Eingegangen bei der Redaktion am 8. Oktober 1940).

Der Häufigkeit zum Trotz, womit die Magenregion in der Strahlentherapie mit Röntgen bestrahlt wird, liegen soweit uns bekannt keine Mitteilungen vor dass klinisch stärkere Schäden im Magen nach der Bestrahlung entstanden sind. *Flaskamp* (1930) kennt in seiner Übersicht über Röntgensschäden keinen klinischen Fall dieser Art. Die meisten klinischen Untersuchungen betreffend die Strahlenreaktion des Magens beschäftigen sich mit den funktionellen Verhältnissen, doch liegen auch einzelne histologische Untersuchungen von Mägen verschiedener Tiere nach Röntgenbestrahlung vor.

Die ersten experimentellen Untersuchungen wurden von *Regaud*, *Nogier* und *Lacassagne* ausgeführt (1912). Sie fanden in 4 Hundemägen nach Röntgenbestrahlung akute und chronische Schäden in der Schleimhaut je nach Dosis und Beobachtungszeit. Sie wiesen darauf hin dass die Hauptzellen der Fundusdrüsen stärker beeinflusst waren als die übrigen Elemente der Schleimhaut. *Ghilarducci* (1914 und 1916) sah Geschwürbildung und Nekrose nach Bestrahlung von Kaninchenmägen. *Tsukamoto* (1924) fand, auch bei Kaninchen, Ulcerationen in der Schleimhaut des Magens nach Leberbestrahlungen, wenn das Bestrahlungsfeld auch Teile des Magens umfasste. Speziell wies er auf eine beträchtliche Entzündungsreaktion um diese Ulcerationen hin. *Dawson* (1925)

und *Wolfer* (1926) bestrahlten beide Hundemägen und fanden Geschwüre; der erste konnte auch volle Heilung feststellen, der andere dagegen hob den chronischen Verlauf dieser Röntgengeschwüre hervor und konnte nur spärliche Bindegewebsneubildung nachweisen. *Engelstad* (1935 und 1938) fand in einem grösseren Kaninchenmaterial Ulcerationen nach der Bestrahlung, in vielen Fällen auch mit guter, sogar vollständiger Heilung und mit Narbenbildung. Er hob ausserdem hervor, dass die Geschwüre am liebsten längs der *curvatura minor*-Seite und in der sogenannten Magenstrasse erschienen. Im Anschluss hieran haben Versuche von *Jano* (1925) spezielles Interesse. Er untersuchte die Heilungsverhältnisse in den verschiedenen Magenteilen des Kaninchens nach künstlich herbeigeführten Geschwüren und bemerkte, dass die Heilung am schlechtesten in der Magenstrasse und um dieselbe herum war, und bedeutend schlechter wenn auch *n. vagus* durchgeschnitten war.

Alle diese Untersuchungen deuten darauf hin dass die Magenschleimhaut moderat radiosensibel ist. Zahlreiche sowohl klinische als experimentelle Untersuchungen von *Heck* (1920), *Mühlmann* und *Meyer* (1923), *Sanders* (1924), *Nánásy* (1935), *Buhtz* (1939), *Gál* (1939) und anderen zeigen, dass die Schleimhaut des Darmes beträchtlich mehr radiosensibel ist. *Witz* (1933) fand Darmreaktion schon nach einer Röntgendosis von 135—140 % einer HED (1 HED = 600 r). In seinen experimentellen Versuche sah *Engelstad* Ulcerationen im Magen des Kaninchens nach 1500 r. *Desjardins* (1931) sagt in einer Monographie über das Verhalten des Magen-Darm nach Röntgenbestrahlung, dass die histologische Röntgenreaktion im Magen in einer Degeneration des Drüsenepithels mit Hyperämie, Leukozyteninfiltration und mehr oder weniger starker Bindegewebsreaktion besteht.

Die durch Röntgenbestrahlung herbeigeführten experimentellen Magengeschwüre sind pathogenetisch nur wenig erforscht. *Engelstad* meint auf Grundlage seiner Untersuchungen, dass die Pathogenese ausser der unmittelbaren Wirkung der Röntgenstrahlen auf der Schleimhaut eine ähnliche wie

für das peptische Geschwür beim Menschen behauptet ist, (chemische, mechanische u. a. Irritanten). Andere Auffassungen sind nicht gefunden.

Zusammengefasst kann man kurz sagen, dass klinische Fälle stärkerer Strahlenschäden im Magen nicht referiert gefunden sind, den Darm betreffend sind dagegen Ulcerationen mit Nekrose nach Röntgenbestrahlung beobachtet. — Experimentelle Untersuchungen über die Reaktion des Magens auf Röntgenbestrahlung zeigen dass die Schleimhaut moderat radiosensibel ist, dass aber bei genügend grossen Dosen Ulcerationen mit Hyperämie, Degeneration des Drüsenepithels, speziell von den Hauptzellen, Ödem und Lenkozyteninfiltration auftreten. Die experimentellen Geschwüre sind dem peptischen Geschwür beim Menschen sehr ähnlich. In einigen Fällen ist Heilung und Narbenbildung beobachtet worden, aber durchweg ist die Regenerationsfähigkeit nur gering.

Die vorliegende Arbeit ist unternommen, um die histiopathologische Entwicklung der Magenulcera zu untersuchen, bei Kaninchen die mit Röntgendosen, die nach *Engelstad* gross genug sind um Geschwüre hervorrufen zu können, bestrahlt sind. Speziell haben wir unsere Aufmerksamkeit auf das Verhalten zwischen Dosis, Grösse und Lage des Bestrahlungsfeldes und die Grösse und Lage des Magengeschwürs gerichtet.

Material, Technik und Methodik.

Für die Versuche sind Kaninchen von einem Monate bis zu ca. einem Jahr benutzt worden, meistens waren die Tiere 7—8 Monate alt bei der Bestrahlung und halten ein Gewicht von etwa 2 kg. Die Bestrahlungen sind mit Siemens Röntgentherapieapparate ausgeführt, die Dosen mit Hilfe eines Mekapion Dosimeters bestimmt worden. Die Strahlenintensivität während der Bestrahlungen war 125r pr. Min. bei 175 KV, 4MA, 3 mm Al-Filter und 18 cm Focus — Hautabstand. Die Bestrahlungsfelder sind von 2×3 bis 4×6 cm, die Strahlenrichtung sowie dorso-ventral wie von Seite zu Seite. Das Feld wurde mit 3 mm Blei abgegrenzt. — Der Magen wurde sofort nach Eintritt des Todes herausgenommen, geöffnet, abgespült und fixiert entweder in 10 % Formol oder Carnoy's Lösung. Die Schnitte sind erstens von Ulcus mit ca. 1 cm von dieser Umgebung heraus-

genommen, zweitens von der Magenstrasse wenn der Magen kein Ulcus oder andere makroskopische Veränderungen zeigte; *Engelstad* stellte nämlich fest, dass die frühesten Veränderungen speziell in dieser Gegend auftreten sollten. Ausserdem sind Schnitte von Fundus und Pylorus herausgenommen. Die Präparate sind nach *Mason's* Trichrommethode, mit Mucikarmin und in speziellen Fällen auch auf Blut nach *Sjöstrands* Methode gefärbt.

Normalanatomische Bemerkungen.

7 Mägen normaler Kontrolltiere sind untersucht worden. Keiner zeigte pathologische Veränderungen, sondern ganz normales Verhalten, in Übereinstimmung mit dem von *Jaffé* (1931) beschriebenen. Doch haben wir unter den Belegzellen im Fundusgebiet, speziell in der oberen Schicht derselben einen charakteristischen Typ Zellen gefunden, der nicht vorher beschrieben worden ist. Diese Zellen sind Belegzellen sehr ähnlich, weichen doch von diesen ab indem sie einen sehr dunklen, kleinen und flachen Kern besitzen, und ein Zytoplasma, das stärker und besser als die übrigen Zellen mit Eosin gefärbt wird. Das Aussehen wird auf Bild 4, die übrige Schleimhaut auf Bild 1—3 illustriert. Die von *Dawson* beschriebenen »Neckzellen« (Nebenzellen) geben positive Mucikarminreaktion. Im Kaninchenmagen sind die Brunnersche Drüsen in Duodenum und die Pylorusdrüsen nicht identisch oder jedenfalls gleich im Aussehen, wie für den menschlichen Magen u. a. von *Meulengracht* (1939) angegeben worden ist. Die Brunnerschen Drüsen reagieren positiv auf Mucikarmin, im Gegensatz zu den Pylorusdrüsen, die sich negativ verhalten.

Der Rauminhalt des Kaninchenmagens ist bei 11 Kaninchen untersucht, und lag zwischen 110 und 220 ccm. Die normale Lage des vollen Magens ist durch Röntgenuntersuchung festgelegt worden. 10 Tiere wurden fotografiert. Sie fasteten 24 Stunden, darauf wurde ihnen 40 ccm Kontrastbrei mittels einer Sonde eingeführt. Der Kaninchenmagen entleert sich erst völlig nach 4—5 Tagen, und ist immer voll wenn genügend zu essen vorhanden ist. 40 ccm ist darum die Menge

PLANCHE I.



Bild 1.
Magenstrasse
(Normalprep.) (60 ×)

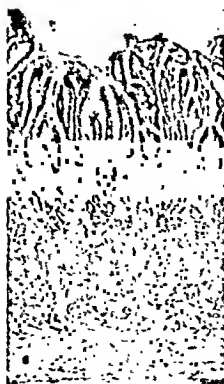


Bild 2.
Pylorus
(Normalprep.) (60 ×)

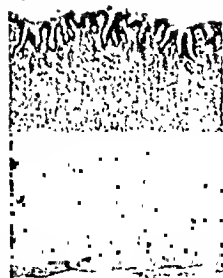
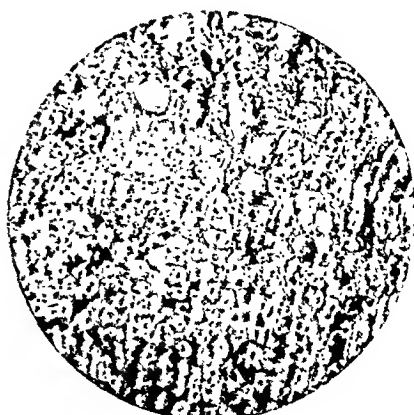


Bild 3.
Fundus
(Normalprep.) (60 ×)



Bild 4.
Der spezielle Typ der Belegzellen (siehe s. 302) in unbestrahltem Magen. (240 ×)



Blid 5.
Dasselbe wie in Bild 4 in bestrahltem Magen. (240 ×)

die vermutlich pro Tag entleert wird. Die Kontrolle dieser Behauptung bei Röntgenaufnahme nach 5 Tagen mit 160 ccm Kontrast war günstig. In Ruhe stimmt die Lage von 9 Mägen gut überein.

Röntgenbestrahltes Material.

Insgesamt sind 71 Tiere röntgenbestrahlt, hiervon bekamen 2 Tiere 2000 r, 47 Tiere 3000 r, 2 — 4000 r, 16 — 5000 r und 4 — 6000 r. Alle Dosen sind stärker als die kleinste Dosis, die nach *Engelstad's* Versuchen genügt, um Geschwüre hervorzurufen. Die Beobachtungszeit war von 24 Stunden bis 162 Tagen. 2 und 2 Tiere sind ungefähr jeden 3. Tag in dieser Versuchsperiode getötet und untersucht worden.

Bei den Untersuchungen der bestrahlten Mägen sind die ersten, groberen, makroskopischen Veränderungen ungefähr 13 Tage nach der Bestrahlung als Erosionen in der Schleimhaut zu sehen. Insgesamt sind 5 Mägen mit Erosionen gefunden, alle im Zeitraum zwischen 13 und 19 Tagen nach der Bestrahlung. Tiefergreifende Defekte, Ulcerationen, wo nicht nur Mucosa, sondern auch Submucosa und Muskularis degeneriert zu finden ist, wurden nicht vor dem 20ten Tag nach der Bestrahlung beobachtet. *Engelstad* hat doch Geschwüre früher gefunden, sogar bereits 7 Tage nach der Bestrahlung. — Das Geschwür zeigt sich makroskopisch als ein runder, gut begrenzter, mehr oder weniger tiefer Defekt in der Schleimhaut. Der Ulcusrand ist mehr oder minder wallförmig verdickt und ist meistens fest infiltriert zu tasten. Die Geschwüre wechseln bedeutend in Grösse, erinnern in Grossen und Ganzen im Aussehen sehr an das peptische Geschwür beim Menschen, ein Vergleich, den u. a. *Engelstad* benutzt. Die Grösse des Geschwürs kann von ein paar Millimetern bis auf 3 cm im Querschnitt wechseln. Nur nach den grossen Dosen sind perforierende Ulcerationen beobachtet worden, insgesamt bei 4 Tieren, in allen Fällen mit Spontan-tod zugehörig.

Von den röntgenbestrahlten Tieren, insgesamt 71, zeigten

PLANCHE II.

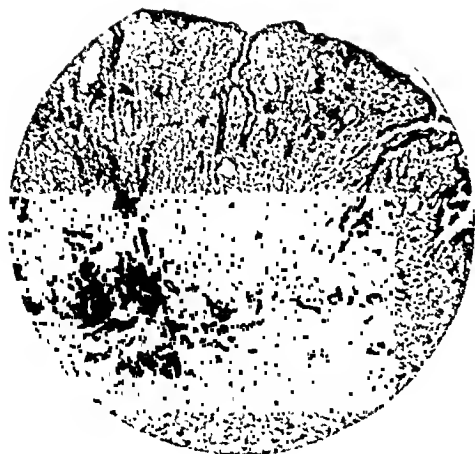


Bild 6.

Rand eines frischen Fundusulcus 22 Tage nach Bestrahlung, mit Degeneration u. Leukozyteninfiltration. (60 \times)



Bild 8.

Schleimhaut aus den Pylorusteil bei Fundusulcus. Drüsen vollständig verschwunden; die Lumina wahrscheinlich Tiefenwucherung des noch erhaltenen Deckepithels. (60 \times)



Bild 7.

Rand eines alten Fundusulcus 97 Tage nach Bestrahlung; Haupt- und Belegzellen vollständig verschwunden; die sichtbare Umwandlung vielleicht regenerativer Art. (60 \times)



Bild 9.

Schleimhaut aus dem Fundus eines Magens ohne Ulcus; vollständige Degeneration der Drüsen; Deckepithel gut erhalten. (60 \times)

52 makroskopische Defekte in der Schleimhaut, davon waren 47 Geschwüre und 5 Erosionen. Von allen Tieren, die länger als 20 Tage beobachtet worden sind, wiesen 11 keine makroskopischen Veränderungen auf.

Um das Verhalten zwischen der Lage der Ulcerationen und der Lokalisation des Bestrahlungsfeldes festzustellen, wurde eine Untersuchung auf Grundlage von Röntgenaufnahmen der Mägen gemacht. In dieser Zusammenstellung sind die Erosionen und Ulcerationen in einer Gruppe zusammengefasst, denn es ist wahrscheinlich anzunehmen, dass die Geschwüre sich auf Grundlage eines Erosionsvorstadiums entwickeln. Diese Zusammenstellung zeigt: In 4 Mägen in denen nur die Pylorusregion bestrahlt ist, wurden 3 Fälle von Pylorusgeschwüre gefunden. Bei 37 Tieren, wo die Bestrahlung gegen den Fundus — Corpusteil gerichtet ist, sind 36 Fälle von Geschwüren in Fundus oder Corpus gesehen. Von 11 Tieren, wo die Bestrahlung grössere Teile des Magens umfasste, sowohl Fundus als Pylorus, sind 4 pylorische Geschwüre und 7 Geschwüre in Fundus zu sehen. Die Pylorusgeschwüre sind meistens in Pylorus selbst zu beobachten, nicht selten greifen sie doch auf den oberen Teil des Duodenumis über. Die Fundusgeschwüre zeigen eine Tendenz Cardia als Prädilektionsstelle vorzuziehen, die Corpusgeschwüre sitzen gern längs der kleinen Curvatur und in der sogenannten Magenstrasse. Nur in zwei Fällen weichen so die Lokalisation der Geschwüre von der Regel, dass sie innerhalb des bestrahlten Magen-gegend erscheinen. In dem einen Fall handelt es sich um Geschwür in Fundusteil nach begrenzter Bestrahlung von der Pylorusregion, in den anderen um Ulcus in Pylorus nach Fundusbestrahlung, in allen übrigen Fällen stimmen Geschwürlokalisation und Feldlokalisation mit einander überein. Könnte man dieser Resultate ganz sicher sein, wäre es verlockend, das Hauptgewicht auf die von der Regel abweichenden Fälle zu legen, man muss jedoch in dieser Zusammenhang darauf aufmerksam sein, welchen Störungen eine exakte Untersuchung ausgesetzt ist, wie z. B. dem Füllungs-

grad des Magens während der Bestrahlung, der Unruhe des Tieres mit Verschiebung des Bestrahlungsfeldes u. s. w.

Auf Grundlage der Gegenüberstellung die wir oben gemacht haben, liegt es nahe den Schluss zu ziehen, dass die hervorgerufenen Geschwüre und Erosionen in den Mägen die Folge einer primären Strahlenschade der Schleimhaut sind. Dies zeigt Übereinstimmung mit der Auffassung von *Engelstad*, dass die Geschwüre in erster Linie ein primärer Strahlenschaden ist, doch behauptet er, dass der Defekt selbst entsteht und unterhalten wird durch chemische und mechanische Irritanten. In Erwägung der im Magen herrschenden komplizierten Verhältnisse und der vielen Möglichkeiten für Einwirkung auf die Schleimhaut auf anderen Wegen ist es wahrscheinlich anzunehmen, dass auch andere Faktoren als die direkte Strahlenwirkung Einfluss auf die Ulcusbildung darstellen. Um nur einige Untersuchungen zu nennen, die dies zeigen, so finden *Ivy*, *McCarthy* und *Orndoff* (1924), *Portis* und *Ahrens* (1924) Säureherabsetzung im Mageninhalt bei Hunden nach Bestrahlung eines Bauchfeldes, das unterhalb des Magens gelegen ist. *Nemenow* und *Jugenburg* (1936) nehmen an, dass die Strahlenwirkung auf den Magen ausschliesslich nervöser Art ist, indem sie in ihrer Röntgentherapie von Magengeschwüre beim Menschen nicht nur lokal bestrahlen, sondern auch einen Teil des Thoracalcolumnas. Dass der Magen auch durch Bestrahlung ausserhalb dieses beeinflusst werden kann, scheint damit bestätigt zu sein, ob aber die Einwirkung so stark ist oder gemacht werden kann, dass Bestrahlung von Hautfeld ausserhalb des Ventrikkelfeldes, oder nach Bestrahlung anderer Organe Ulcerationen oder Erosionen hervorrufen, oder dazu mitwirken kann, ist noch eine offene Frage, auf die man, obwohl sie sehr wohl einer Untersuchung wert sein könnte, kein Antwort in dieser Arbeit erhält. —

In unseren Versuchen haben wir Ulcerationen von verschiedener Grösse, von ein paar Millimeter bis auf 3 cm im Querschnitt gefunden. Wir haben einen Vergleich zwischen Ulcusgrösse und Feldgrösse gemacht, finden aber keine Über-

einstimmung hierin; ein kleines Feld kann ebensogut ein grosses Geschwür hervorrufen, wie ein grosses Feld einen kleinen Ulcus. Die Zeit scheint auch keine Rolle zu spielen, natürlich abgesehen von der Zeit, in welcher das Geschwür gebildet wird, diese Zeit ist doch wahrscheinlich ziemlich kurz, nur 1—2 Tage. Es ist nicht mit Sicherheit eine stufenweise Abstossung des degenerierten Gewebes, wie es *Dahl* (1937) für das Röntgeschwür der Haut angibt, zu bemerken. Eine Progression des Geschwüres mit der Zeit ist nicht gefunden. Die benutzte Röntgendosis spielt auch keine wesentliche Rolle, doch sind die Geschwüre nach den grösseren Dosen durchweg etwas grösser als nach den kleineren. *Engelstad* hebt auch diese Unabhängigkeit der Geschwürgrösse von der Grösse des Bestrahlungsfeldes hervor, nach den Feldgrössen, die von 3×4 bis 6×8 cm wechseln, findet er dennoch Geschwüre von ungefähr derselben Grösse, nämlich ca. 1 cm im Querschnitt.

Bei den makroskopischen Untersuchungen der Mägen nach Bestrahlung sind in vielen Fällen schon nach 30 Tagen, und in allen Fällen nach 60 Tagen Adhäsionen von Dickdarm, Dünndarm oder Oment zu Serosa dem Geschwüre entsprechend zu sehen, selbst wenn der Prozess nicht die Muscularis durchbricht und die Serosa direkt affiziert, ein Verhalten, das auch beim Magengeschwür des Menschen wohlbekannt ist. Die Verwachsungen sind weniger ausgesprochen nach den grossen Dosen, was sich leicht erklärt durch schlechtes Regenerationsverhalten. —

Die histologische Untersuchung worauf das Hauptgewicht gelegt ist, geht in Kürze darauf aus, den Prozessen zu folgen, denen die Schleimhaut nach Röntgenbestrahlung ausgesetzt ist.

Die ersten Veränderungen sind schon nach 24 Stunden sichtbar. Wir finden da die Kapillargefässe in der bestrahlten Gegend des Magens erweitert und mit Blut gefüllt, ein Verhalten das schon von *Engelstad* bemerkt worden ist. Nach und nach verbreitet sich diese Hyperämie oder Erythem zu immer grössere Teilen des Magens, sodass wir nach 13 Tagen

Hyperämie praktisch innerhalb des ganzen Magens sehen können. Das Erythem ist immer selbst nach den längsten Beobachtungszeiten zu sehen, doch am stärksten um die Geschwüre herum. Erinnern wir an das Verhalten in der Haut, so ist die Röntgenreaktion gewöhnlich in ein Früherythem und ein Späterythem geteilt worden, die doch beide auf das bestrahlte Gebiet begrenzt sind. Beide Reaktionen sind von mehreren Untersuchern, als eine Freiwerdung der sogenannten H-Substanz, eines histaminähnlichen Stoffes, zu erklären versucht. *Engelstad* nimmt an, dass die Frühreaktion in der Schleimhaut, also nach 24 Stunden, derselben Natur ist, und dieselben Ursachen hat, wie das Früherythem in der Haut. Ob das Früherythem in der Schleimhaut des Magens abklingt, ehe die Späthyperämie einsetzt, wissen wir nicht. Die Späthyperämie im Magen steht dadurch im Gegensatz zum Späterythem in der Haut, weil die Ersten meistens zu ganzen oder grossen Teilen des Magens verbreitet zu sehen ist, selbst wenn das Bestrahlungsfeld sehr klein gewesen ist (2×3 cm). *David* (1926) fand nach Bestrahlung der Schwimnhaut des Frosches eine starke Einwirkung auf die kontraktiellen Elemente der Gefässwände, die Rougetschen Zellen, eine Manifestation, die nur funktionel nachweisbar war, histologisch konnte er keine sichere Veränderungen in den Gefässen feststellen. Es liegt darum nahe, diese Späthyperämie als eine kombinierte Wirkung auf die kontraktiellen Elemente der Kapillarwand und Freiwerdung von H-Substanz wegen der verbreiteten Hyperämie, speziell weil wir histologische Veränderungen in den Gefässen nur im späteren Stadien, erst nach ca. 40 Tagen nach der Bestrahlung beobachten können. — Die Hyperämie ist der Ausdruck einer Zirkulationsstörung, und es liegt nahe anzunehmen, dass diese eine Rolle für die Ulcusbildung spiele. *Engelstad* hat in einem Fall nach Tuschinjektion um ein Ulcus schon nach 10 Tagen eine Infarktähnliche Zone beobachten können, und wirft damit die Frage auf, ob nicht Gefässveränderungen und damit Zirkulationsstörungen eine mitwirkende Ursache für die Ulcusbildung darstellen.

Ödem tritt wie auch das Erythem sehr früh nach der

Bestrahlung auf, leichtes Ödem ist schon nach 24 Stunden in dem bestrahlten Gebiet des Magens zu sehen, und breitet sich nach ca. 5 Tagen über die ganze Schleimhaut. Später ist es ein konstanter Fund, doch am stärksten in den früheren Stadien des Ulcus, und am deutlichsten in den Fällen wo auch Perforation eintritt. — Im Anschluss an das Ödem, aber ein wenig später erst nach 7—8 Tagen nach der Bestrahlung, ist eine leichtere Leukozyteninfiltration in der Schleimhaut zu sehen. Nach und nach zeigt sich das Bild einer gewöhnlichen Gastritis, gewöhnlich begrenzt zum bestrahlten Teile des Magens, aber auch diffus. Beim Erscheinen der ersten Erosionen, nach etwa 13 Tagen, ist die Entzündung um diese stark. Die Leukozyteninfiltration besteht zunächst hauptsächlich aus Lymphozyten, später aus grösseren Mengen Granulozyten und Plasmazellen. Die Gastritis nimmt mit der Ulcusbildung wesentlich zu, und ist am stärksten etwa 40 Tage nach der Bestrahlung, hat jedoch innerhalb unserer Versuchszeit keine auffallende Tendenz wieder abzunehmen. Der Wundrand ist hauptsächlich von Lymphozyten und Plasmazellen infiltriert, unterhalb des Geschwüres und im Geschwürboden sind mehr eosinophile und pseudoeosinophile Granulozyten zu sehen, meistens nur eine kleine Anzahl von Fibroblasten. Die Befunde stimmen in dieser Weise mit dem Verhalten bei Gastritis und Ulcus im menschlichen Magen gut überein.

Bei Untersuchung der verschiedenen epithelialen Elemente der Schleimhaut nach der Bestrahlung sind schon nach 5 Tagen leichte degenerative Veränderungen im Deckepithel im bestrahlten Gebiet des Magens zu sehen. Die Änderungen äussern sich durch Vakuolen in das Zytoplasma und Pyknose der Kerne. Nach der Bildung des Ulcus ist das Deckepithel in der Umgebung oft ganz destruiert oder zeigt starken Verfall. Es ist in dieser Weise kein Unterschied zwischen Pylorusgeschwüren und Fundusgeschwüren. Auch in den übrigen Teilen des Magens weist das Deckepithel Degeneration auf, ein Umstand, den man natürlich mit der verbreiteten Gastritis in Zusammenhang setzt. Um alte Ulcerationen herum geht

das Deckepithel meistens ganz bis zum Rande, es ist daher mit der Möglichkeit einer Regeneration zu rechnen. —

Wie es bei dem Deckepithel der Fall ist, zeigen sich schon nach 5 Tagen erkennbare Veränderungen degenerativer Art in den Pylorusdrüsen und Hauptzellen der Fundusdrüsen. Für die Belegzellen der Fundusdrüsen sind die ersten Veränderungen schwerer festzulegen, doch kommen sie später zum Ausdruck, und sind weniger ausgesprochen als bei den Hauptzellen, ein Umstand der schon von *Regaud*, *Nogier* und *Lacassagne* beobachtet und später von u. a. *Engelstad* bestätigt worden ist.

Um die Ulceration herum ist zum Teil vollständige Degeneration des Drüsenepithels zu sehen, sowohl bei Fundus- als bei Pylorusgeschwüren, doch scheinen die Pylorusdrüsen weniger radioempfindlich zu sein als die Hauptzellen der Fundusdrüsen.

Die früher beschriebene Art der Belegzellen zeigt, wie aus Bild 5 hervorgeht, eine auffallende Vermehrung nach der Bestrahlung, während die Nebenzellen gewöhnlich ganz verschwunden oder nur spärlich zu sehen sind. *Dawson* nimmt an, dass eben diese Zellart, weil sie um die Drüsenmündungen gelegen ist, die Regenerationsstelle der verschiedenen Elemente der Schleimhaut bildet.

Ein etwas eigenartiges Phänomen, das oft, besonders in den späteren Stadien der Geschwüre zur Erscheinung kommt ist eine Umwandlung der Drüsen in den nächsten Umgebungen des Geschwüres. Wir finden hier cystisch erweiterte Hohlräume mit einem kubischen Epithel bekleidet. Die Kerne in diesen umgewandelten Drüsenzellen sind gross, rund und erinnern nicht wenig an die Kerne der Belegzellen. Das Cytoplasma gibt schwache Mucikarminreaktion, was übrigens keine anderen Elemente in der normalen Schleimhaut als die Nebenzellen tun. Eine ähnliche Umwandlung der Drüsen ist auch bei Geschwüre in Pylorusregion in der Pylorusschleimhaut zu sehen. Das Deckepithel zeigt oft eine Tendenz zu Tiefenwuchs um ältere Geschwüre herum und mit Drüsenähnlichen Abschnürungen in der Tiefe. *Hauser* 1926) wies eine

eigenartige Metaplasie und Tiefenwuchs der des Drüsenepithels in der Schleimhaut um das gewöhnliche Ulcus Pepticum beim Menschen hin, ein Umstand den er nicht näher zu erklären versucht. Es ist möglich dass hier eine Übereinstimmung besteht, und dass eigentlich nicht die Bestrahlung diese Veränderungen hervorruft, sondern die Ulcusprozess selbst und die Reaktion der Schleimhaut mit Gastritis und Regeneration. Man muss daher annehmen, dass diese Metaplasie von sowohl Drüsenepithel als Deckepithel in Wirklichkeit der Ausdruck für eine Regenerationstendenz der Schleimhaut ist. Wie früher erwähnt ist dies auch speziell in den späteren Stadien des Ulcus zu beobachten.

Die Regenerationsfähigkeit der Schleimhaut nach den hier angewandten Dosen ist sehr gering, nur spärliche Bindegewebsneubildung und Fibroblastreaktion ist zu sehen. Dies stimmt mit Beobachtungen von *Dawson* und *Wolfer* gut überein, die auf Grundlage ihrer Untersuchungen hervorliehen, dass das Röntgenmagengeschwür als ein chronisches Geschwür zu betrachten sei, und meinten dass es für Studien über das chronische, peptische Geschwür beim Menschen geeignet wäre. In unserem Versuche ist kein Fall von Narbenbildung oder voller Heilung beobachtet worden; man muss jedoch auf die begrenzte Versuchszeit, 150 Tagen, erinnern; es ist wohl möglich dass Heilung noch später eintreffen kann, was *Engelstad* gefunden hat.

Interessant sind die Befunde bei 11 Tieren die nach 2000, 3000 und 5000 r keine Geschwüre zeigten. 2 davon waren nur 1 Monat alt bei der Bestrahlung. Von 4 Tieren, die 1 Monat alt bestrahlt worden waren bekamen nur das Eine Ulcus, dieses Tier war dabei das einzigste von Allen, das deutliche Zeichen von Regeneration aufwies, der kurzen Beobachtungszeit, 42 Tagen, zum Trotz. Bei den anderen 3 dieser Gruppe wurden keine Geschwüre festgestellt, auch hatten sie nur spärliche Degeneration in der Schleimhaut. Dies ist wahrscheinlich ein Ausdruck dafür, dass die Regenerationsfähigkeit bei jungen Tieren bedeutend stärker ist, als es für erwachsene und ältere gibt. Die übrigen 9 Tiere die keine Ulcerationen oder Ero-

sionen aufwiesen, zeigen doch deutliche degenerative Veränderungen im bestrahlten Gebiet der Schleimhaut. Dieses fehlende Auftreten von Geschwüren spricht für eine individuelle Variation der Strahlenempfindlichkeit, die häufigen Observationen in der Klinik dementsprechend. Eine Kontrolle des richtig gelegenen Bestrahlungsfeldes haben wir in den epilierten Hautfeldern. — Ein Umstand von wesentlicher Bedeutung ist der starke Einfluss auf das Allgemeinbefinden der Tiere nach Magenbestrahlungen, und der es verschieden zu sein scheint, je nachdem die Bestrahlung gegen Pylorus gerichtet ist oder als sie die anderen Magenteile betrifft. Als Mass für das Allgemeinbefinden der Tiere ist das, immer wieder kontrollierte Gewicht benutzt worden. Vergleichen wir diejenigen Tiere mit Ulcus in Pylorus oder Magenstrasse, die einen Gewichtsverlust von $\frac{1}{2}$ Kg. oder mehr haben, mit den Tieren mit Ulcus im Fundus oder Corpus, können wir folgendes feststellen: Von 38 Tieren mit Ulcus in Fundus-Corpus starb nur 1 spontan und 8 hatten Gewichtsabnahme von mehr als $\frac{1}{2}$ Kg. Von 15 Tieren mit Geschwüren in Pylorus oder Magenstrasse starben 4, und 8 hatten denselben Gewichtsverlust, also 24 — und 80 % stark beeinflusstes Allgemeinbefinden beziehungsweise für Fundusgeschwüre und Pylorusgeschwüre. Es geht ein deutlicher Unterschied aus diesen Zahlen hervor. Den Grund dieser starken Beeinflussung des Allgemeinbefindens der Tiere ist es nicht möglich ohne weitere Untersuchungen klar zu legen, es soll nur erwähnt werden, dass in 2 Fällen von Pylorusulcus eine hochgradige Anämie beobachtet worden ist.

Zusammenfassung.

71 Kaninchen sind in die Magenregion röntgenbestrahlt, und in verschiedenen Beobachtungszeiten von 24 Stunden bis auf 162 Tagen untersucht worden. 2 Tiere sind ungefähr je 3 Tage getötet worden. Die Schleimhaut ist histologisch untersucht, und die Befunde können wir kurz in folgenden Sätzen aufsummieren:

Röntgenbestrahlung von Kaninchenmägen nach Dosen von 2000—6000 r ruft Degeneration der Schleimhaut meistens mit Geschwürbildung hervor.

47 Tiere hatten Geschwüre, die frühesten nach 20 Tagen nach der Bestrahlung, während Erosionen schon nach 13 Tagen auftraten.

Die Grösse der Geschwüre hat keine Relation zur Feldgrösse oder Zeit. Sie sind durchweg etwas grösser nach den grossen Dosen als nach den kleineren. Mit den angewandten experimentellen Verhältnissen ist die Übereinstimmung zwischen Geschwürlokalisation und die Lage des Bestrahlungsfeldes offenbar.

Die Strahlenreaktion ist bedeutend stärker in den Hauptzellen als in den Belegzellen.

Ein spezieller Typ der Belegzellen ist gefunden worden. Sie haben ein stark eosinophiles Cytoplasma und einen kleinen, stark befärbten Kern. Sie sind sowohl in bestrahlter als unbestrahlter Fundusschleimhaut zu sehen, aber deutlich vermehrt nach der Bestrahlung.

Morphologische Gefässveränderungen kommen erst in den späteren Stadien des Ulcus vor, und auch dann spärlich.

Regeneration ist nach den hier angewandten Dosen und Beobachtungszeiten nicht oder nur wenig zu sehen. Bei sehr jungen Tieren dagegen ist die Regenerationsfähigkeit bedeutender.

Das Allgemeinbefinden ist gewöhnlich stark beeinflusst, stärker, wenn das Ulcus in Pylorusteil des Magens sitzt als in den übrigen Teilen.

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UPSALA.

SPECIFIC PULMONARY ALTERATIONS IN TUBEROUS SCLEROSIS.

By *Gert Vejens.*

(Received for publication October 8th 1940).

The term tuberous sclerosis is generally taken as meaning the same thing as tuberous sclerosis of the brain. *Berg* and *Vejens*, however, have already dissociated themselves from this view and have expressed their adherence to *Josephy's* opinion that tuberous sclerosis is a malformational disease of a general character and not a specifically cerebral affection. Our opinion is supported by observations made by us on a case, published last year, of tuberous sclerosis of the brain occurring concomitantly with a peculiar pulmonary disease that pointed both clinically and anatomically to 'cystic lung', but which in certain marked respects was of another nature and revealed a tendency to tumorous formation. The disease we have described appears to manifest itself familiarly, as tuberous sclerosis of the brain often does. On this point we are continuing the investigation. The account of the disease, already given by us, I wish here to illustrate by a somewhat more detailed report on the pathologico-anatomical findings — partly in order to show that we are in fact concerned with a case of tuberous sclerosis of the brain and partly to establish the specific character of the pulmonary alterations.

Of the *clinical data* I will mention here only the following: The patient, an unmarried woman, was healthy until she reached the age of 30. Round the mouth and nose, however, she had had, since the age of 17 or 18, numerous small yellow and light red nodules. The disease broke out in an acute form with a spontaneous pneumothorax. Subsequently the subject's condition alternated between periods of pneumothorax and to all appearances perfect health. Gradually it became apparent that a pulmonary heart disease had developed, combined with reduced vital capacity of the lungs (1500 c.c.). There were no indications of psychic illness. After approximately two years of clinically manifest disease symptoms, the patient died at the age of 32.

Pathologico-anatomical findings.

An external examination of the body revealed, around the nasal alae and in the naso-labial sulci, numerous pale red or yellow-green nodules, about the size of a pin-head or somewhat smaller. No microscopic investigation was made of these '*Pringle's naevi*'. There was nothing else of importance to be noted. Thus the structure of the body was normal and the musculature and flesh not noticeably reduced.

On the exterior of the *cerebrum* no alterations were visible. On the convexity of the frontal lobes, on the other hand, and in the adjacent parts of the parietal and temporal lobes, there were numerous palpable resistances resembling tumours and ranging from pea to hazel-nut size. These were diffusely limited and were of a fairly firm consistency. These foci exhibited a characteristic appearance under the microscope, corresponding to the descriptions first given by *Bourneville*. The alterations, however, were but slightly pronounced. The outermost layer of the cerebral cortex was interlaced with hypertrophic neuroglia tissue, consisting chiefly of glia-fibres parallel to the surface proceeding from an increased number of glia-cells, a portion of which were atypical and very often large in size (fig. 1). In the immediately successive layers of the cerebral cortex the numerically increased glia-fibres formed a thin network, which occurrence represents a deviation from their normal presentation. It was not only in the surface strata but also in the subcortical medulla that the glia-cells showed a numerical increase, the cells being atypical quite often and very large (fig. 2). They frequently contained several large, more or less irregularly formed nuclei.

In general the architectonic of the cerebral cortex with *Brodman's* characteristic strata of differently sized pyramidal cells etc., was well preserved even in the tuberous foci. In certain cases, however, these revealed a completely irregular structure. They then

contained, within the regions which may be assumed to correspond to the 3rd and 4th layers, a great number of abnormally large,

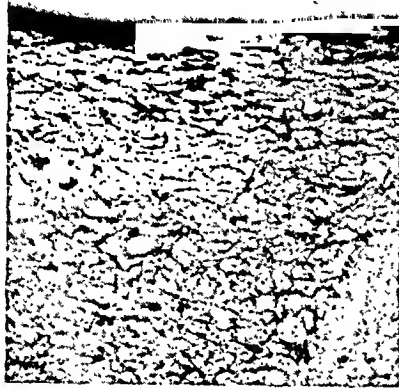


Fig. 1.

Neuroglia proliferation at the surface of a cerebral tuberos formation.

irregularly formed and unsystematically directed pyramids, which were all the more in evidence in that the total number of nerve-cells was considerably reduced (fig. 3). The medullary sheaths showed a clear numerical increase in the superficial tangential layer.

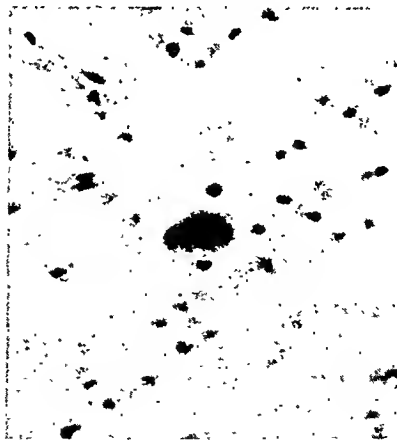


Fig. 2.

Glia-cell of abnormal form and size in the subcortical medulla.

The left cerebral hemisphere contained on the surface facing the ventricle two greyish white, firm tumours, rather less than bean size. The microscope revealed that these tumours were gliomas with focalic calcifications.

Even if in this case the pathologico-anatomical cerebral alter-

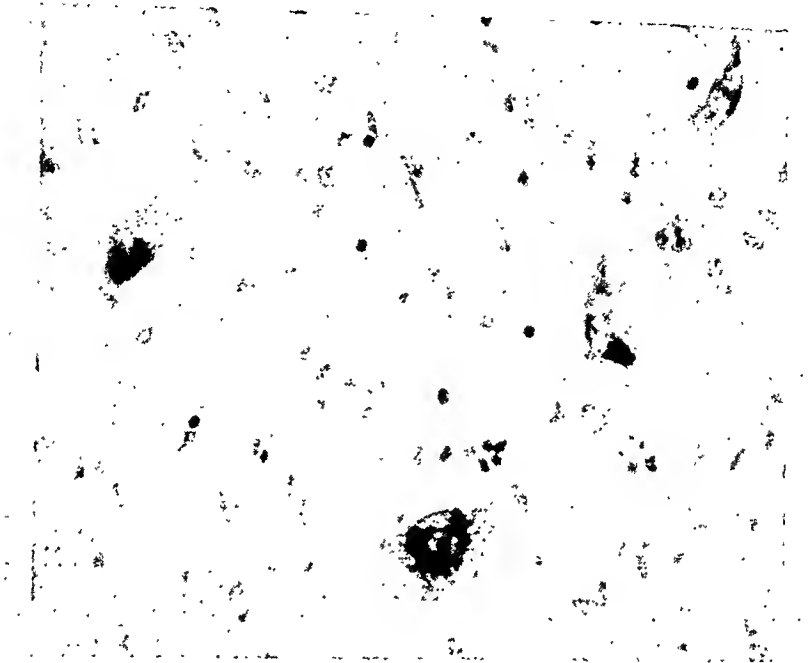


Fig. 3.

Atypical pyramid cells in a tuberos focus.

ations are only moderately pronounced, we may nevertheless regard them as satisfactory evidence of a *tuberos sclerosis of the brain*.

The *heart* was large and dilated and its musculature hypertrophic. The alterations affected in particular the right half, agreeing with an impeded circulation in the pulmonary circuit. The musculature was diffusely indurated with connective tissue but the valves and coronary arteries showed no alterations. No tumours were visible.

The *lungs* presented a unique appearance. They were everywhere, except laterally at the right tip, ongrown to the wall of the thorax by means of rather loose connective adherences. The free space corresponded to a pneumothorax cavity of the dimension of a good-sized tangerine, the existence of the cavity having been previously

röntgenologically attested. The lungs were large and voluminous and felt to be more air-cushioned than usual. The surfaces were dotted with small air-filled bubbles, not more than pea-size, suggesting a bulbous emphysema.

The cross section was greyish brown in colour like a stasic

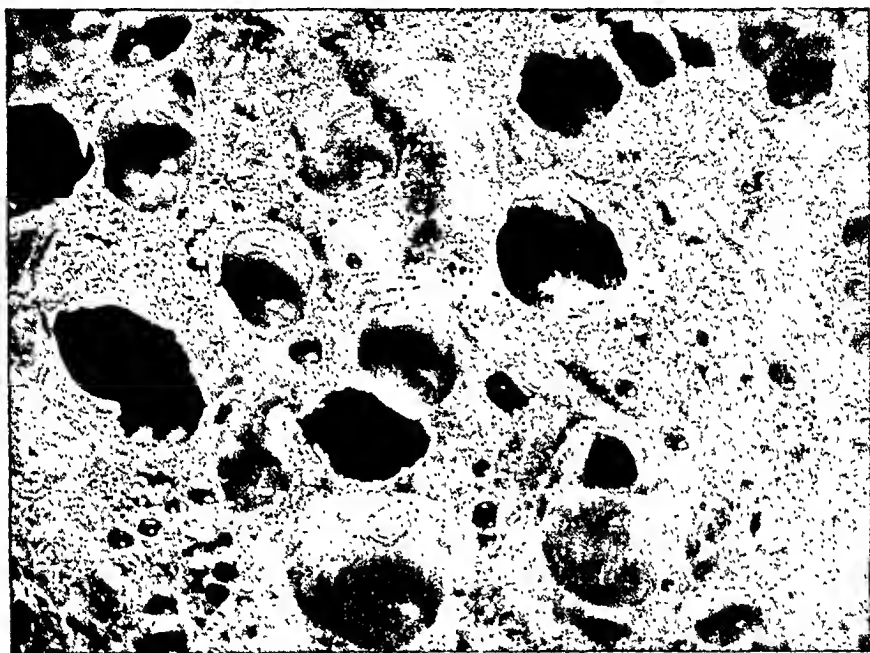


Fig. 4.

Cross-section of the lung showing the cystic malformations.

lung. It presented an appearance totally different from normal lung tissue, resembling a bath-sponge or a honeycomb which agreed with the puzzling indications of the previously made x ray (fig. 4). It was diffusely cribbled with small cavities embedded in compact tissue. These cystic formations contained air, and here and there by exception, a clear, serous fluid; they never exceeded pea-size and the smallest ones were too minute for observation with the naked eye. The walls between the larger cysts often reached $\frac{1}{2}$ cm. in thickness, but they then contained a number of miniature cysts. In places the cyst walls were only the thickness of paper and had been perforated, so that here and there systems of confluent cysts,

separated by imperfect septa, were formed. One was unable to detect any ordering of the cysts along the ramifications of the bronchial tree, but in various places it was apparent that open communication existed between them and the finer bronchial tubes. The inner surface of the cyst walls was glossy and smooth but in the majority of the cysts numerous greyish white nodules, up to pin-head size, were to be seen marking the surface. Similar small, tumour-like formations were apparent in many places embedded in the septa.

Microscopic examination showed that only insignificant traces remained of normal lung parenchyma. The picture presented was of multitudinous cyst-like cavities of every conceivable size, separated by walls of greatly varying thickness. The majority of the cysts were round in form, whilst others were infallen like empty sacks or spread out into ramifications. A lesser number of the cavities were wholly, or within small sections of the walls, covered with cylindrical bronchial epithelium and can thus be taken to correspond to the pulmonary cysts described by *Oudendal* and others as caused by an arrested development of the bronchi in the embryonic phase. The big majority of the cystic cavities were, on the contrary, without epithelium covering. Towards the lumen, however, much flattened cells were revealed which were here and there rather like alveolar epithelium. It is not clear how far these cavities, without or with an uncharacteristic cell covering, are to be regarded as congenital cystic formations or as emphysematic bubbles originating during the post-foetal life and the problem can hardly be solved by the deductions from a single section. It seems, nevertheless, reasonable to assume that the majority of them, even though undergoing alterations during life both as to form and size, are in fact congenital cysts.

The question of the possible congenital origin of the 'cysts' is, however, in the present case of less importance than the question of the character of the interstitial lung tissue. The walls of the cysts consist to a large extent of connective tissue containing numerous blood-vessels. But the most salient component is plain muscular tissue (fig. 5). This muscular tissue, moreover, is not, as in the descriptions of cystic lung previously given by *Buchmann* and *Oudendal* and others, disposed in thin superficially parallel layers. Nor does it bear resemblance to the more irregular muscular proliferations of *von Stössel's* muscular cirrhosis of the lung. It has the unmistakeable character of *tumorous tissue*, a fact which differentiates the present case of '*cystic lung*' from all other cases described hitherto. The nuclei of the muscular fibres are, in the main somewhat broader than usual. Further the contractile sub-

staneæ, which is coloured yellow in the normal way by pieric acid, according to *van Gieson's* method, has not always the same regular narrow fusiform shape, which is so characteristic of this smooth museulature. The museular fibres are often shorter and thicker and blunted at the ends. They are probably to be regarded as



Fig. 5.

Myomatous walls between pulmonary eysts.

survivals from the embryonic stage, without however presenting the appearance of malignant tumour cells. The essential characteristic of this myomatous tissue is that the muscle fibres are joined together in intercrossing bunches and clusters precisely as in the case of an ordinary myoma (fig. 6) and in the same way as in the tumours frequently observed in tuberous sclerosis, or the tumour-like formations in the kidneys for example (cf. figs. 7 A and B). The lungs thus present, apart from the general cystic transformation, the aspect of a diffuse *myomatosis*, which must be considered as very closely related to the genuine tumour formation.

In the interstitial lung tissue, furthermore, the usual indications of a stasis were observed with hemorrhages and the accumulation

of iron-content pigment. No inflammatory alterations were apparent of any mentionable importance. The elastic fibres were clearly reduced in number but the reduction was not striking. This alteration may be considered as having contributed to a development and an increase in size of the cyst-like cavities. It

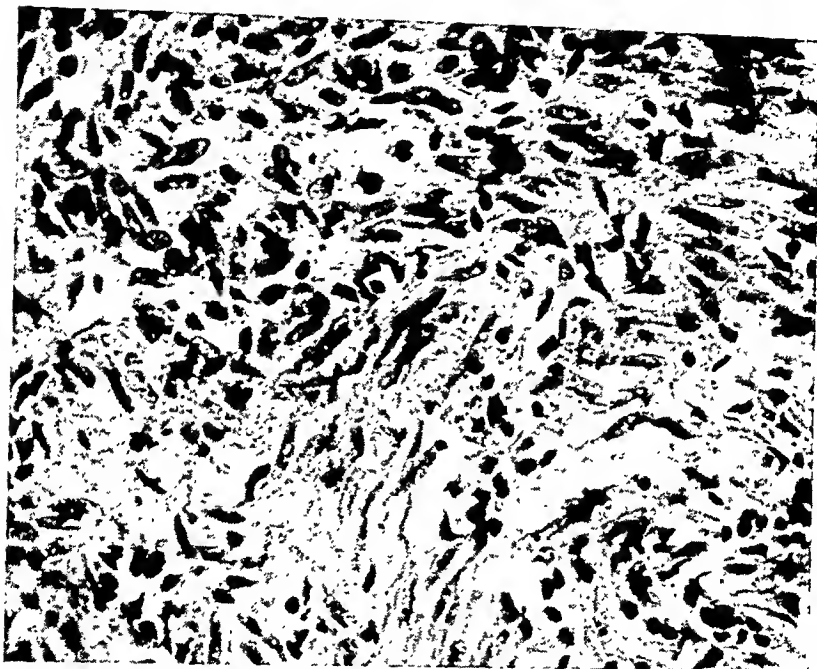


Fig. 6.

Leiomyoma (myoblastoma) in the lung.

can scarcely, however, have been of any essential importance, in that it was insufficiently pronounced.

The *kidneys* were of approximately normal size. They lay for the most part embedded in a conglomerate of partially intergrowing greyish white tumours, varying from pea-size to the size of a plum. All these tumours were situated outside the renal capsule. They deformed the kidneys but their growth only encroached on them at individual places. Everywhere in the cortex and here and there in the medulla the cross section was full of similar tumours up to pea size. Otherwise the renal parenchyma, apart from stasis symptoms, exhibited no obvious alterations.

The *renal* and *pararenal* tumours presented in the main the same appearance. As was the case with the pulmonary tumours,

they were formed of plain embryonic muscular tissue growing, as in a myoma, in intercrossing clusters of muscle fibres. These were quite short and at times contained almost round nuclei (fig. 7 A). In the myomatous tissue, as at places in the renal parenchyma, small lipomata or lipoma-like malformations were intercalated, often traversed by passages of plain musculature (fig. 8). It would ap-

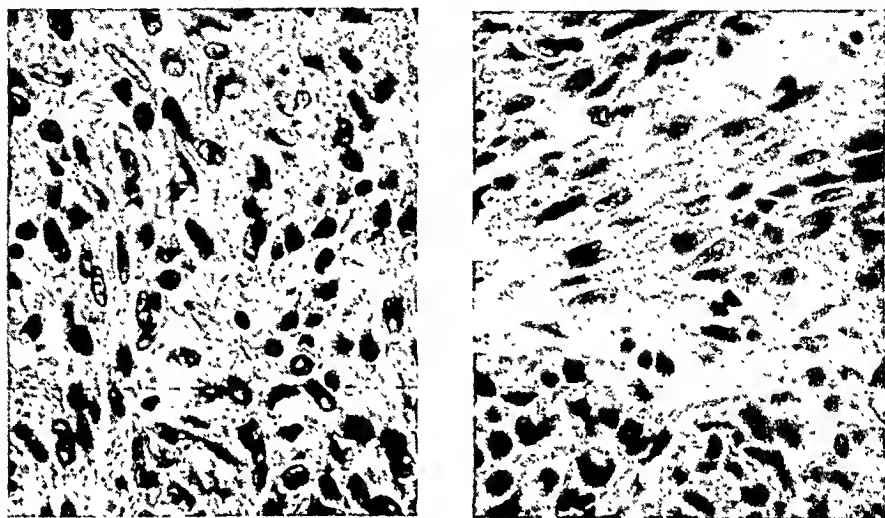


Fig. 7.

Myomata, A in the renal capsule, B in a lymphatic gland.

pear to be a matter of taste whether these phenomena are best defined as multilocular benign tumours — leiomyomata, non-striated myoblastomata, lipomata, lipo-myoblastomata — or as tumour-like malformations. 'Mixed tumours' of this and of similar types in, and especially around, the kidneys occur concomitantly, as has frequently been observed before, with tuberous sclerosis of the brain. It may be mentioned incidentally that in the kidneys, especially just beneath the surface, individual cysts covered with cubic epithelium were encountered.

In this connection it is perhaps worth recording as a curiosity that on investigating the parathyroidal glands on the left side of the trachea behind the thyroid gland, a roundish formation was encountered somewhat larger than a peppercorn and of a greyish-white colour. Microscopic investigation was necessary before it could be identified, whereupon it proved to be a *lymphatic gland*. Of the glandular tissue, however, only a very small portion re-

mained. The greater part of the gland was transformed into an *embryonic myoma* or, if the term be preferred, a myoma-like malformation partly surrounded by intracapsular lipomatous tissue (fig. 9). Its appearance under the microscope corresponded in all

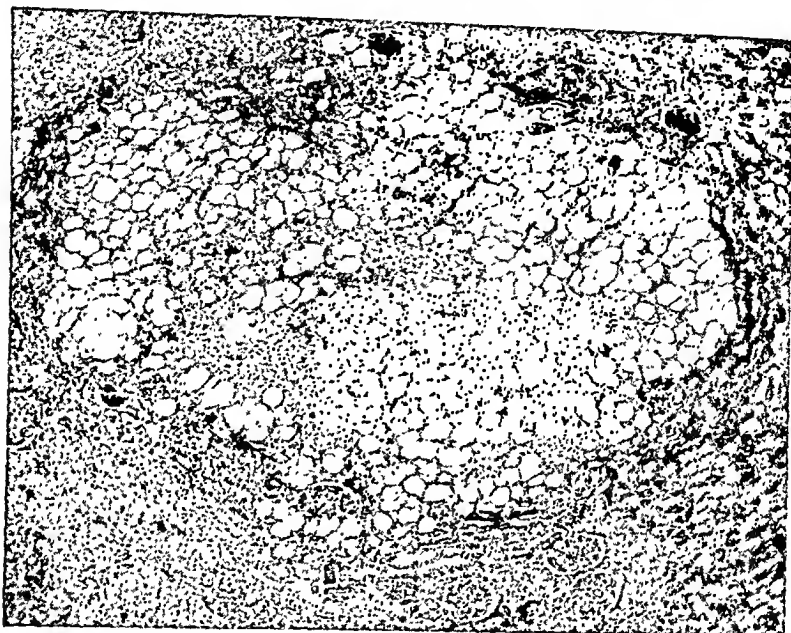


Fig. 8.

Lipo-leiomyoma (lipo-myoblastoma) in the kidney.

essentials with the renal and pulmonary tumours already described (fig. 7 B).

The *spleen* was somewhat enlarged and exhibited symptoms of chronic stasis with induration of the tissue. It contained at the lower pole a fairly well delimited tumour about egg size, of a firm consistency, with light reddish grey, even sectional surface. The microscope revealed a tumour of comparatively rare occurrence, a *splenoma*, formed of oval reticular cells. It is to be remembered that the splenoma — even though it is commonly called a primary splenic tumour — is generally considered as intermediary between a tumour and a malformation.

The *liver* exhibited a chronic stasis but was not remarkable otherwise.

The *endocrine glands* contained no tumours but presented a number of striking alterations which may well be of significance

as regards the origin and progress of the disease. In the hypophysis a foecaliform hyperplasia of the principal cells was found, especially in the anterior lobe, and a clear diminution of the eosinophile cells. In the thyroidal and parathyroidal glands there were no certain pathological alterations. The suprarenals were

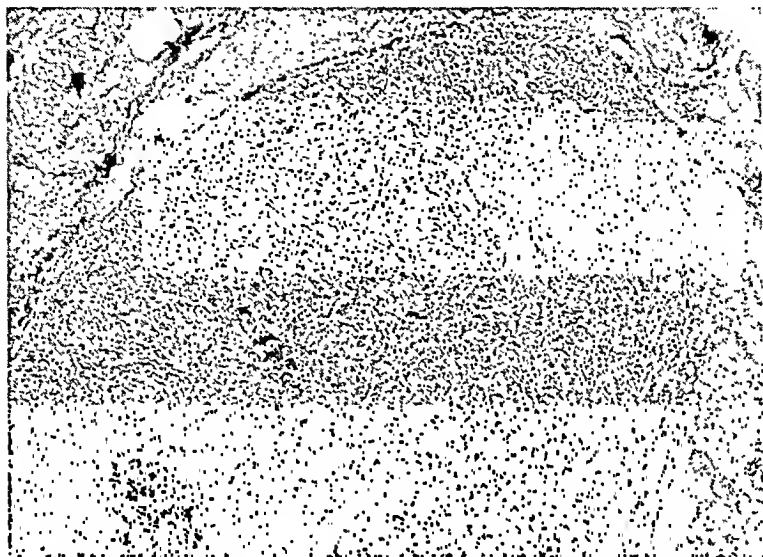


Fig. 9.

Lymphatic gland containing a myoma and, to the right, lipomatous tissue. On the left and below remains of lymphatic tissue.

small, 2—3 cm. in length and only a couple of millimeters in thickness. The cortex was of normal appearance but the medulla was atrophied in a high degree. The ovaries were atrophic and their cortex ovarially deficient.

Discussion.

The above account is in the main a re-affirmation simply of already well-known alterations in cases of tuberous sclerosis. Thus the cerebral and renal alterations do not afford any new data of exceptional interest. The myoma in the lymphatic gland and the splenoma, however, are in the nature of novelties. But what is of interest in the present case is

the *specific pulmonary alterations* and their *familiar occurrence*. The deceased patient left a sister and two brothers, all of whom are still alive. In the case of the younger sister, characteristic röntgenological alterations of the lungs, as well as *Pringle's naevi* and the papillomatous growths at the nails so typical of tuberous sclerosis, have been constated. The three brothers in the family are reported to have similar facial naevi. A clinical investigation of them will be undertaken, if circumstances permit, in the near future. In this connection the röntgenological results will be published by Dr. *Berg* and Dr. *Zachrisson* in *Acta radiologica*.

Recently *Bruce* has published an analytical survey of the various types of cyst-affected lung of congenital origin, in which congenital cystic lung is represented as being formed of thin-walled cysts. It may happen that the cyst-walls burst, giving rise to a spontaneous pneumothorax. This, however, presupposes that the cysts are in open communication with some bronchial branch. In other cases the impeded circulation of the blood gradually gives rise to pulmonary heart disease. In the case described by *Berg* and myself, both these complications manifested themselves. A third complication is, according to *Bruce*, an infection which on account of inflammatory infiltration together with proliferation of the connective tissue and musculature, causes a thickening of the cyst-walls, a circumstance which renders röntgenological diagnosis difficult.

But the case here described shows that the thickening of the cyst-walls may be caused in another way, i.e. by an infiltration of tumorous or tumour-like tissue. It is doubtful whether the lung cysts in this case should *per se* be characterized as malformations having no essential connection with the formation of tumours, or as an expression of tumour formation. But if one adopts the view that tuberous sclerosis is intermediary between a tumour formation and a malformation, and if one knows that sclero-tuberous alterations were established in the brain and the kidneys as usual, and were also found in a single lymphatic gland and in the spleen,

and if, further, due regard is paid to the fact that the disease is of familiar character, then one is justified, it would seem, in holding that the *myomatous alterations in the lung tissue* are also an expression of a *general malformational disease with a tendency towards tumour formation*. Whether the case is then to be designated as cystic lung or not, can be regarded as a matter of opinion. For my own part, I regard the alteration as a *tuberous sclerosis of the lungs*, or, expressed in another way, as a tuberous sclerosis with the essential alterations localized in the lungs and with secondary symptoms of the same nature in the brain and kidneys etc.

On microscopic examination of the endocrine organs, certain clear alterations were observable in the case of some of them, *e. g.* a reduction in number and size of the eosinophile cells in the pituitary gland, and an atrophy of the ovaries and of the medulla of the suprarenals. These alterations are possibly of importance for the explanation of the origin of tuberous sclerosis and the understanding of the genesis of tumours in general. But in view of the uncertainty of the evidence adduced from the investigations hitherto undertaken on alterations of the endocrine glands in cases of cancer and other tumorous diseases, I deem it inexpedient at the present moment to go into the question of the possible connection between tuberous sclerosis and malignant tumours and a disturbance of the functioning of the endocrine glands. I am, nevertheless, prone to believe that such a connection does in fact exist.

Summary.

Last year *Berg* and *Vejlens* published an account of an exceptionally rare disease, which was of familiar occurrence but whilst presenting the usual symptoms of a tuberous sclerosis of the brain, was in other respects peculiar. The essential alterations, of which a pathologicco-anatomical description was given, were not localized in the brain but in the *lungs*. These showed a general cystic degeneration and

— what is most remarkable — in the septa of the cysts a myomatous proliferation of a tumorous character. The altered lungs, accordingly, should not be regarded as ‘cystic lung’ of a new type but as one of many expressions of a malformational disease with a tendency towards tumour formation. The alteration is here designated as ‘tuberous sclerosis of the lungs’.

The genesis of the disease is not clear. That the alteration is of congenital origin may, however, be regarded as certain. Further, co-present alterations in the histology of certain endocrine organs point to some connection between the tuberous sclerosis and a disturbance of equilibrium in the endocrine system.

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ON THE DEVELOPMENT OF ANTIBODY IN RABBITS IMMUNIZED WITH HUMAN BLOOD CORPUSCLES OF TYPE A.

By *Jorgen Flamand Christensen.*

(Received for publication November 5th, 1940).

Immunization of rabbits with human blood corpuscles of type A has shown that there is a great difference between the different animals with regard to the development of antibody reacting with that A antigen which characterizes this blood type.

This was for the first time demonstrated by *Hooker & Anderson* who, in 1921, found that only 2 out of 5 rabbits immunized with human blood corpuscles of type A responded by producing specific agglutinin.

Numerous investigators have carried on these studies and obtained interesting results, which illustrate both the composition and occurrence of the A antigen and the conditions which involve variations in the individual development of antibody in response to the introduction of the same antigen.

Thus *Schiff & Adelsberger* in 1924 found that animals responding to immunization by developing A agglutination, at the same time produced antibody which, in the presence of complement, hemolysed sheep red corpuscles, and which caused precipitation with alcoholic extract of guinea pig kidneys. They showed thereby that A antigen is contained in sheep red corpuscles and in organs of guinea pigs, whence

this A antigen must belong to that group of »heterogenetic« antigens which are termed *Forssmann antigens*. However, with the aid of absorption experiments they likewise showed that this Forssman antigen only represents part of the A antigen of human red corpuscles. *Schiff* straightway calls this part of the A antigen »Schafanteil« (sheep part), because it is also contained in sheep red corpuscles, whereas he calls the remaining part of the A antigen »Restanteil« (remainder).

These experiments were subsequently confirmed by numerous investigators (*Dölbers, Witebsky, Okabe, Mai, Hara, Thune Andersen*). However, by a special examination of the A antigen content of sheep red corpuscles *T. Andersen* has shown that this »Schafanteil« cannot be considered to be a well defined unity, for he demonstrates that the quantity of A antigen in sheep corpuscles varies greatly and in such a manner that a certain part of the A antigen is contained in the red corpuscles of *all* sheep, whereas the red corpuscles of *some* sheep *in addition to it* contain a smaller or greater quantity of A antigen. Moreover, I have shown that the A antigen which — as a species property — is contained in the red corpuscles of all sheep is essentially the same as that contained in the organs of guinea pigs, thus being a Forssman antigen proper. On the other hand, that A antigen which is contained in the red corpuscles of some sheep only, is not detectable in the organs of guinea pigs.*)

*) *T. Andersen* has shown that this difference in the antigen content of sheep corpuscles is due to a number of qualitatively different fractions of A antigen, which may be contained in these corpuscles. By absorption of immune sera, produced by immunization of rabbits with human red corpuscles of type A, with blood corpuscles of different sheep he found that the red corpuscles of some sheep only removed a minor part of the sheep hemolysin, whereas others removed a greater part of it. This is due to a qualitative difference in the A antigen content of sheep red corpuscles. Part of the A antigen — as a species property — is common for all sheep but, besides this part, the red corpuscles of some sheep contain one or several qualitatively different A components.

This difference in the A antigen content of sheep red corpuscles

As was mentioned before, rabbits immunized with human red corpuscles of type A are not all capable to develop antibodies, but whereas the majority of investigators are of opinion that rabbits either do develop antibodies reacting with all the components of the A antigen or do not develop any A antibody at all, *O. Thomsen* partly on the ground of theoretical reflections thinks that such a sharp differentiation is not correct. Thus he mentions for example that the serum of virtually all rabbits contains a spontaneously developed A agglutinin which is of very varying strength however. Experiments of immunizing rabbits with A corpuscles accordingly reveal that A agglutinin is developed in all the animals, whereas there seems to be a sharper differentiation between them with regard to their property of producing sheep hemolysin.

The object of the experiments, which shall be described in the following, is to clear up these conditions, that is to say, to demonstrate what rabbits after immunization with human red corpuscles of type A do react by developing antibodies, and whether the antibodies react with the entire A antigen or only with fractions of it.

In another paper will be described the constitutional conditions which are at the bottom of the different modes of reaction.

Forty rabbits altogether were immunized with A_1 corpuscles and 14, with A_2 corpuscles. The antibody formation being the same whether A_1 or A_2 corpuscles were used for the immunization, the two groups will be described collectively. Some animals only received 3 injections at intervals of 2 days, whereas the immunization of others was continued for up to 7 months, the first injections being given at intervals of a couple of days, and later — when the quantity of antibody had ceased to increase — at intervals of from 1 to 2 weeks.

must, perhaps, be considered an analogy to the difference in the A antigen content of human red corpuscles, which manifests itself by the existence of the so-called subgroups.

For the injections was used a 10 % suspension of blood corpuscles in physiologic saline, 1 cc. of which was injected into an ear vein. Anaphylactic reactions were never observed after the injections. The antibody formation was controlled with the aid of frequent examinations performed both prior and subsequent to the immunization. Every examination included determination of the quantity of *species-specific antibody* by agglutination with corpuscles of type O. This antibody was subsequently removed by absorption with blood corpuscles of this type. Afterwards the content of A *agglutinin* and *sheep hemolysin* was determined. This latter determination was made with sheep red corpuscles containing much A antigen. Then the serum was absorbed with sheep red corpuscles containing very little A antigen, and finally the serum was subjected to examination in order to detect whether it still contained hemolysin opposite sheep red corpuscles containing much A antigen. In that way it was endeavoured to ascertain whether the serum only contained hemolysin opposite that A antigen which exists in the red corpuscles of all sheep, or whether it contained at the same time antibody opposite such A antigen as is found in some sheep only.

Agglutination experiments were performed with the technic usually applied in this institute, quantitative determinations being made by titration of 0.1 cc. of serum in physiologic saline, the first of a series of diminutive test-tubes containing 0.1 cc. of undiluted serum, whereas in the following tubes was placed 0.1 cc. of fluid containing $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$ etc. volume of serum, respectively. To each tube was then added 0.1 cc. of an about $\frac{1}{2}$ % suspension of blood corpuscles. After vigorous shaking the tubes were left to stand at room temperature, and readings were made as a rule after about 18 hours. Occasionally the readings were made after the lapse of 4 hours, because hemolysis appeared in isolated sera when the tubes were left to stand longer. Comparative readings after the lapse of 4 and of 18 hours did not reveal any essential difference in the magnitude of the titer. For these experiments fresh or, at the highest, 24 hour old corpuscles were used. In numerous cases it was necessary to dilute the serum to titration, dilutions of $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$ etc. then being used in conformity with those obtained by titration.

In the subjoined tables recordings are made in plus signs, +++ implying very strong agglutination with coarse lumps and coherent

clouds, ++ strong agglutination with coarse granules, + distinct agglutination with fine granules, and (+) implying traces of or doubtful agglutination. The agglutination titer is expressed by the reciprocal value of the weakest serum concentration which is able to agglutinate blood corpuscles, that serum concentration being proceeded from, which the tubes contain before the suspension of blood corpuscles is added. Thus, even though the serum is diluted before titration, the titer will always be a power of 2.

The quantity of hemolysin was determined quantitatively by titration in the same way as the agglutinin. As diluter was used diluted guinea pig complement (cpl.). In the first tube was placed 0.2 cc. of cpl. diluted with physiologic saline in the proportion of 2/15, in the following tubes, 0.2 cc. of cpl. diluted 1/15, in which 0.2 cc. of serum (eventually serum dilution) is titrated. Thereby a complement dilution of 1/15 is obtained in all the tubes, and a serum dilution of 1/2 in the first tube and of 1/4, 1/8 etc. in the following tubes.

After titration 0.05 cc. (1 drop) of a 5 % suspension of sheep red corpuscles is added to each tube, and after vigorous shaking the tubes are placed on a constant-water-bath of 37° for 20 minutes.

Readings are made when the nonhemolysed corpuscles have settled down. The degree of hemolysis is estimated roughly according to the coloration of the fluid and the size of the precipitate, and it is recorded with the numbers 100 — 80 — 60 — 40 — 20 — 0, which represent different percentages of total hemolysis.

Just as in agglutination the hemolysis titer is expressed by the reciprocal value of the lowest serum concentration which is able to give unquestionable though faint hemolysis, no regard being paid to the slight dilution brought about by the addition of blood corpuscle suspension.

The complement fixation experiments were performed with the technic devised by O. Thomsen, which is usually applied in this institute: 0.1 cc. of serum is titrated in a series of tubes containing 0.1 cc. of saline each so as to obtain the dilutions 1/2, 1/4, 1/8, etc. To these dilutions is added 0.1 cc. of the antigen solution and 0.1 cc. of cpl. solution containing 1½ titer dose. After 1½ hour's standing at room temperature is added 0.2 cc. of a 2.5 % suspension of sheep red corpuscles sensitized with 2½ dose of ambocceptor; then the tubes are placed on a constant-water-bath for 20 minutes, and readings are made in the same manner as in the hemolysis experiments. Control experiments with regard to the autofixation of serum and antigen are performed at the same time.

These systematic examinations showed that rabbits according to their capability of developing antibodies could be divided into two well characterized groups, and this difference was quite independent of their having been immunized a few times or during a very long period.

This is illustrated by the graphs, Figures 1, 2, 3, and 4, which show the quantities of the various antibodies found in 4 rabbits (R 14, R 1, R 15, and R 27). The titer, recorded as a power of 2, which corresponds to the number of tubes with positive reaction, is noted on the ordinate, whereas the time, distributed into weeks, is noted on the absciss-axis. The dates recorded on the latter represent the days of examination, whereas the vertical lines indicate the days of immunization.

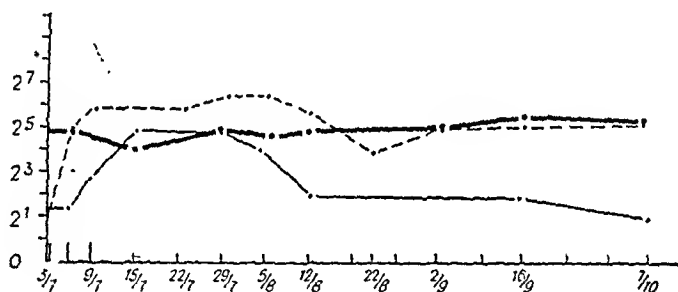


Fig. 1.

R 14, immunized 3 times with A_1 blood corpuscles.

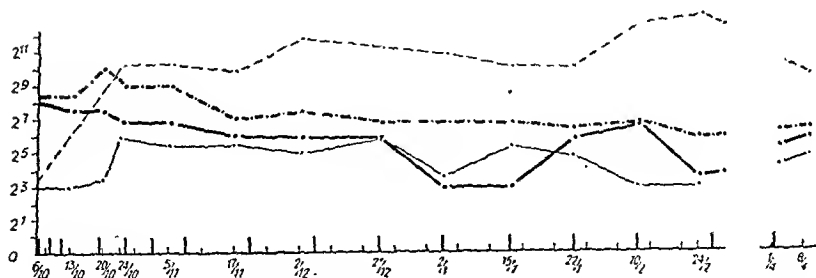


Fig. 2.

R 1, immunized with A_1 blood corpuscles in the course of 6 months.

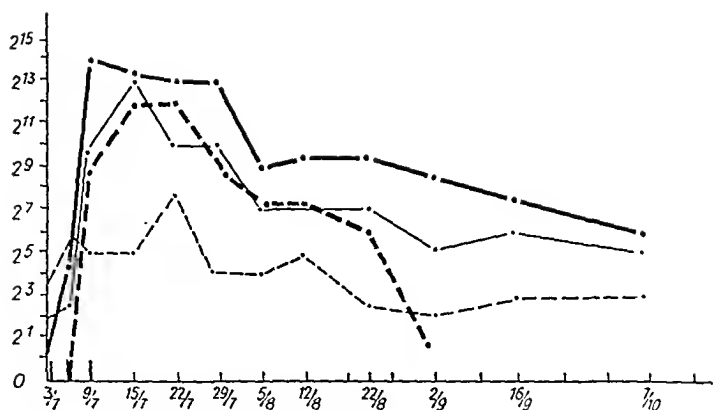


Fig. 3.

R 15, immunized 3 times with A₁ blood corpuscles.

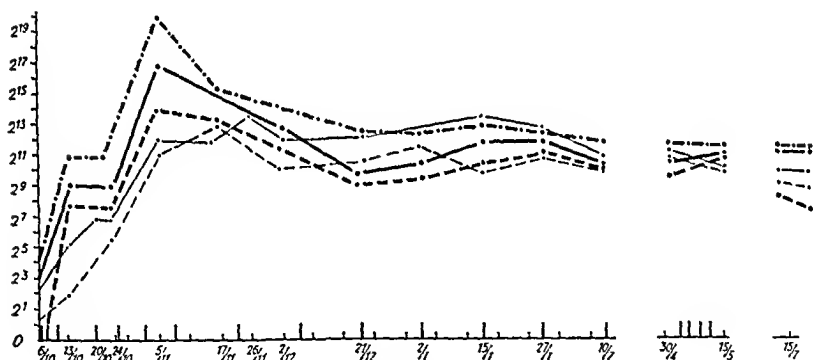


Fig. 4.

R 27, immunized with A₁ blood corpuscles in the course of 7 month.

Agglutination of O blood corpuscles: — — — — —

Agglutination of A₁ blood corpuscles; serum absorbed
with O corpuscles: — — — — —

Hemolysis of sheep red corpuscles; serum not absorbed: — . . . — . . .

Hemolysis of sheep red corpuscles; serum absorbed
with O corpuscles: — — — — —

Hemolysis of sheep red corpuscles; serum absorbed
with O corpuscles, and with sheep corpuscles
with little A antigen: — — — — —

The two rabbits, R 14 and R 15, were immunized with 3 injections made at intervals of 2 days, whereas the other two, R 1 and R 27, were immunized frequently throughout 7 months.

The figures show a distinct difference in the capability of the rabbits of developing antibodies, R 14 and R 1 having produced only a very small quantity of A antibody, whereas R 15 and R 27 have developed antibodies with a high titer.

Comparison of the several curves traced for these 4 animals shows a distinct elevation of the titer for agglutination of blood corpuscles of type O. The elevation is smallest in the animals immunized a few times, though there is no essential difference between the 4 rabbits.

Comparison of the curves representing the quantity of A agglutinin shows, however, only a slight elevation in R 1 and R 14, the titer for the A agglutination being constantly lower than the titer for the O agglutination. In contradistinction to this, a very considerable increase of the quantity of A agglutinin, which exceeds the quantity of O agglutinin, is found in R 15 and R 27.

A still more pronounced difference is evidenced by the curves traced for the sheep hemolysin. These titration curves show that, in R 1 and R 14, there does not ensue any increase whatever of the hemolysin existing prior to immunization, whereas a considerable increase of it is observed in R 15 and R 27. On examining particularly whether, after absorption of serum with sheep red corpuscles containing very little A antigen, there is hemolysin corresponding to that A antigen which is contained in the blood corpuscles of some sheep only, it becomes evident that such hemolysin is at no time detectable in serum from R 1 and R 14, whereas, after immunization, it is detected with a high titer in R 15 and R 27. This hemolysin, which fails to be detected in serum before immunization, also disappears entirely some time after the termination of the immunization, whereas the other antibodies gradually return to their initial values.

These 4 rabbits are typical examples of the differences in the property of rabbits to develop antibodies. That the same differences assert themselves in all the remaining rabbits is shown by Table 1 and Table 2, which jointly comprise all those animals which were immunized with A blood corpuscles.

These Tables record (1) the highest titers for agglutination of O blood corpuscles, (2) the titers for agglutination of A corpuscles prior to immunization as well as the highest titers after immunization, (3) the corresponding titers for the hemolysis of sheep red corpuscles and, finally, (4) the corresponding titers for the hemolysin corresponding to that A antigen which is found in some sheep only.

In Table 1 are recorded all those animals which had but little capability to produce antibodies, whereas the others are recorded in Table 2.

On glancing at these tables it is seen that the titers for the *agglutination of O corpuscles* vary from 2^5 to 2^{15} . *O. Thomsen* mentions that those rabbits which produce strong A antibody develop but little O antibody, whereas, reversely, those which produce little A antibody form much O antibody, for he thinks that the production of O antibody is inhibited by the strong A antibody formation. However, even though my own experiments show that the lowest anti-O titer is found in an animal which produced strong A antibody, and the highest titer in an animal which produced only a small quantity of A antibody, the two tables on the whole show such uniform values that there is no clue for this assumption. It has indeed been found that, if rabbits are immunized with blood corpuscles of type O, they will certainly all produce antibody in profusion but still in distinctly varying quantities. Thus the capability of producing A antibody cannot alone be decisive of the quantity of O antibody which develops after immunization with A blood corpuscles.

On the other hand, Table 1 and Table 2 show a pronounced difference between the animals with regard to the *A agglutinin*.

Table 1.

Rabbit Nr.	Agglutination of O blood corpuscles	Agglutination of A blood corpuscles. Serum absorbed with O corpuscles		Hemolysis of sheep corpuscles. Serum absorbed with O corpuscles		Hemolysis of sheep corpuscles. Serum absorbed with O corpuscles, and with sheep corpuscles with little A antigen	
	after immunization	before immunization	after immunization	before immunization	after immunization	before immunization	after immunization
1	2 ¹³	2 ³	2 ⁷	2 ⁸	2 ⁷	0	0
26	2 ¹⁵	2 ¹	2 ⁷	2 ⁴	2 ⁶	0	0
43	2 ¹¹	2 ¹	2 ⁴	2 ⁷	2 ⁶	0	0
45	2 ¹²	IIA 1	2 ²	2 ¹	2 ⁴	0	0
47	2 ¹²	2 ¹	2 ³	2 ⁶	2 ⁶	0	0
51	2 ¹³	IIA 1	2 ⁴	2 ⁷	2 ⁵	0	0
52	2 ¹⁰	IIA 1	2 ³	2 ³	2 ⁷	0	0
14	2 ⁶	2 ²	2 ⁵	2 ⁵	2 ⁵	0	0
16	2 ⁷	2 ³	2 ⁵	2 ⁴	2 ⁵	0	0
31	2 ¹⁰	1	IIA 2 ²	2 ⁸	(2 ⁹)	0	0
34	2 ⁹	2 ²	IIA 2 ²	2 ⁴	(2 ⁹)	0	0
35	2 ⁸	2 ³	2 ³	2 ⁵	(2 ⁸)	0	0
37	2 ⁹	2 ¹	2 ³	2 ⁶	(2 ⁷)	0	0
38	2 ³	2 ³	2 ⁴	2 ⁶	(2 ⁸)	0	0
39	2 ¹¹	2 ¹	2 ⁴	2 ⁵	(2 ⁹)	0	0
41	2 ¹⁰	2 ³	IIA 2 ³	2 ⁵	(2 ⁹)	0	0
5	2 ¹⁵	2 ⁴	2 ⁵	2 ⁶	2 ⁵	0	0
18	2 ⁹	2 ¹	2 ⁴	0	0	0	0
19	2 ¹³	2 ¹	2 ³	2 ³	2 ⁴	0	0
54	2 ¹⁰	2 ¹	2 ³	2 ⁶	2 ⁵	0	0
55	2 ¹²	2 ¹	2 ¹	2 ⁷	2 ⁷	0	0
56	2 ⁹	1	2 ⁴	2 ⁶	2 ⁵	0	0
60	2 ¹¹	0	2 ²	2 ⁷	2 ⁶	0	0
61	2 ¹⁰	2 ¹	2 ²	2 ⁵	2 ⁴	0	0
62	2 ¹²	2 ²	2 ⁴	2 ⁷	2 ⁵	0	0

Table 1 shows the highest values of the different antibody determinations before and during the immunization. It comprises those rabbits which had but little capability to produce antibodies. The first 7 rabbits were immunized with from 11 to 18 injections, the next 9, with from 3 to 5 injections of A₁ blood corpuscles, 7 of these animals had shortly before been immunized with sheep red corpuscles, whence they had formed sheep hemolysin. Immunization with A blood corpuscles caused no further increase. The remaining 9 rabbits were immunized with A₂ blood corpuscles.

Table 2.

Rabbit Nr.	Agglutination of O blood corpuscles	Agglutination of O blood corpuscles. Serum absorbed with O corpuscles		Hemolysis of sheep red corpuscles. Serum absorbed with O corpuscles		Hemolysis of sheep red corpuscles. Serum absorbed with O corpuscles, and with sheep red corpuscles with little A antigen	
	after immunization	before immunization	after immunization	before immunization	after immunization	before immunization	after immunization
3	213	23	213	27	214	0	210
8	213	22	214	26	216	0	213
22	214	26	215	211	215	0	213
24	213	24	214	26	215	0	212
27	213	23	214	23	217	0	214
42	28	23	29	25	214	0	213
44	29	21	212	27	216	0	214
46	25	VII 1	213	28	217	0	216
48	28		212	28	215	0	214
49	27	VII 1	210	27	215	0	214
50	28		211	25	214	0	212
57	211	21	211	29	216	27	214
58	210	21	29	26	215	0	213
12	27	23	211	21	210	0	210
13	28	26	212	25	210	0	29
15	28	22	213	1	214	0	212
20	28	25	211	27	211	0	210
21	26	23	29	26	214	0	210
23	27	23	29	24	214	0	211
28	28	25	29	24	214	0	211
32	26	23	210	25	211	0	29
33	27	24	29	26	215	0	29
36	27	25	212	27	215	0	212
40	26	25	210	28	216	0	214
4	210	23	211	25	216	0	213
11	29	27	210	26	213	0	211
17	212	26	210	29	212	0	212
53	211	22	210	26	214	0	210
59	28	21	29	25	212	0	29

Table 2 shows the highest values of the different antibody determinations before and during the immunization. It comprises those rabbits which produced strong antibodies. The first 13 animals were immunized with from 11 to 18 injections, the next 11 animals, with from 3 to 5 injections of A₁ blood corpuscles, and the last 5 animals were immunized with A₂ blood corpuscles.

The animals in Table 1 after immunization present titers of from 2^1 to 2^7 , that means to say, there are some in which no increase is detected after immunization despite these animals (R 31, R 34, R 55) having been immunized with 4 injections made in the course of 10 days. In other animals is found a distinct though, in all these cases, rather slight increase.

However, all the rabbits recorded in Table 2 after immunization present a distinct titer elevation. The elevation certainly varies but it is in all the animals distinctly greater than in the rabbits of Table 1.

In the columns 5 and 6 of the tables are recorded the titers for *sheep hemolysin*, which show a fundamental difference between the two groups. A single case (R 26) in Table 1 shows a very slight increase of titer which is, moreover, only detected by a single examination shortly after the beginning of the immunization. In none of the other animals of this group is an increase of the sheep hemolysin found. However, in 7 of these animals the hemolysin titer is found to be higher after the immunization than in the normal serum. But that is due to the circumstance that these animals had previously been immunized with sheep red corpuscles and, hence, developed sheep hemolysin which had not quite disappeared when they were immunized with A blood corpuscles. Since the quantity of hemolysin was not increased by the immunization with A blood corpuscles, and as it was not decreased by absorption with A blood corpuscles, it may be presumed that these animals have not produced hemolysin opposite that A antigen which is contained in sheep red corpuscles.

On the other hand all the animals in Table 2 present a very pronounced and strong elevation of the titer of sheep hemolysin.

This difference in the antibody formation of the two groups is still more pronounced in the last two columns which show the quantity of antibody opposite the A antigen found in the red corpuscles of some sheep only. Such anti-

body is extremely seldom found to be preformed in rabbit serum. *T. Andersen* declares that he has never detected it, whereas it was found in one of my rabbits (R 57). This antibody is produced by all those animals which form a considerable quantity of A agglutinin and sheep hemolysin, whereas the animals of the other group do not produce it at all.

Thus it can be asserted that the rabbits according to their antibody formation after immunization with blood corpuscles of type A can be divided into two distinctly separate and almost equally large groups: The animals in the one group produce no or very little A agglutinin and no sheep hemolysin, whereas those of the other group produce strong A agglutinin as well as strong sheep hemolysin, and this sheep hemolysin consists both of antibody opposite the A antigen contained in the blood corpuscles of all sheep, and opposite that which is only found in some sheep.

The reason why this distinct difference has not been emphasized by previous authors probably is that regard is but seldom paid to the circumstance that the formation of A antibody is accompanied by synchronous formation of species-specific antibody, and that this latter may also contain sheep hemolysin. Thus, if rabbits are immunized with O blood corpuscles, the great majority of them will develop sheep hemolysin. Therefore it is necessary to absorb serum with O blood corpuscles, before the antibody corresponding to the A antigen is determined, for otherwise the quantity of hemolysin corresponding to antigen which is common to sheep and all human beings regardless of the blood type will be determined at the same time. For example in my experiments a fall of the titer for sheep hemolysin from 2^{11} to 2^7 was found after absorption with O blood corpuscles.

As was mentioned before, almost all the animals produce A agglutinin, those of the one group producing but little, and those of the other group producing much.

This A agglutinin is an expression of the total quantity of A antibody which the animal is capable of producing, and therefore it must, in those animals which can produce sheep hemolysin, consist partly of antibody opposite the A antigen contained in sheep red corpuscles. On the other hand, in those animals which cannot produce sheep hemolysin the A agglutinin cannot contain antibody opposite the A antigen of sheep red corpuscles.

The experiment recorded in Table 3 illustrates this. The two rabbits, R 1 and R 24, were immunized regularly for 7 months, and the table shows experiments performed, partly, prior to and partly during the immunization. Obviously both R 1 and R 24 have produced A agglutinin after immunization. By absorption of serum with blood corpuscles from two sheep whose blood corpuscles contain little and much A antigen, respectively, a considerable part of the agglutinin is removed in serum from R 24, whereas no agglutinin is removed in serum from R 1. As is seen in Table 1 and 2, R 1 could not produce sheep hemolysin, whereas R 24 produced strong sheep hemolysin.

Now it might be supposed that the difference in the quantity of A agglutinin, which is found between the two groups, were due solely to the different capability of producing antibody opposite the A antigen of the sheep red corpuscles, while there should be no difference in the capability of producing antibody opposite the remaining part of the A antigen.

In order to detect this it was endeavoured to remove all the antibodies opposite the A antigen in sheep red corpuscles by absorbing serum with sheep corpuscles having as great an A antigen content as possible. As is evident from the experiment recorded in Table 3, the titer was thereby lowered in those animals which produced strong antibody, so considerable a difference in the quantity of A agglutinin persisting however as to suggest that the various response of the rabbits does not only depend of the capability of producing antibody opposite the A antigen contained in sheep red corpuscles but

Table 3.*

Date	1/4	1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096
13/10:	R24 abs. with O " " " + S49 ~ " " " + S61 ~	+	+	+	+	+	+	+	+	+	+
15/1:	R24 abs. with O " " " + S49 ~ " " " + S61 ~	-	-	-	-	-	-	-	-	-	-
8/4:	R24 abs. with O " " " + S49 ~ " " " + S61 ~	-	-	-	-	-	-	-	-	-	-
13/10:	R 1 abs. with O ~ A1: " " " + S49 ~ " " " + S61 ~	+	(+)	(+)	0	0	0	0	0	0	0
5/11:	R 1 abs. with O ~ " " " + S49 ~ " " " + S61 ~	+	+	+	+	+	+	+	+	+	+
27/1:	R 1 abs. with O ~ " " " + S49 ~ " " " + S61 ~	+	+	+	+	+	+	+	+	+	+

Table 3 shows the quantity of A agglutinin in serum from two rabbits (R24 and R 1) both prior to immunization and at different times during it. By absorption with sheep red corpuscles an essential part of the agglutinin in the serum of R 24 was removed, but none in that of R 1.

*) In Table 3 and Table 4 are used abbreviations, for example: R24 abs. with O+S49 ~ A1, which means that serum from rabbit 24 is absorbed with human O blood corpuscles and with sheep red corpuscles from sheep 49, and that it is subsequently employed in the upper horizontal line represent the serum dilutions. A1 blood corpuscles. The fractions noted in the upper horizontal line represent the serum dilutions.

also of the greater or lesser capability of producing antibody opposite the remaining part of the A antigen.

As was previously mentioned, *Schiff & Adelsberger* detected that the antigen produced by immunization with A blood corpuscles causes precipitation in an alcoholic extract of guinea pig kidney; it was further demonstrated that the A antigen which is thus contained in guinea pig kidneys is identical with that contained in the red corpuscles of all sheep. Therefore it is natural that, after immunization with A blood corpuscles, only those rabbits which produce sheep hemolysin also produce antibody opposite guinea pig kidney extract. This is illustrated by the experiment recorded in Table 4, which shows that R 49 after immunization has produced strong A agglutinin as well as sheep hemolysin and antibody which gives fixation of the complement with guinea pig kidney extract. R 59 on the other hand has produced weak agglutinin only, and neither sheep hemolysin nor complement-fixing antibody.

It might be expected that the pronounced difference in the capability of producing antibody opposite the A antigen of sheep red corpuscles became manifest not only after immunization with human blood corpuscles of type A but also after immunization with sheep red corpuscles. However, *Hirszfeld* reports that he has more rarely observed rabbits producing A agglutinin after immunization with sheep red corpuscles than after immunization with human corpuscles.

In order to clear up this question 20 rabbits were immunized with sheep red corpuscles. As was anticipated all the animals produced strong sheep hemolysin, but in 12 of them part of the hemolysin could be removed by absorption with A blood corpuscles, and these 12 actually produced A agglutinin at the same time. However, this agglutinin was not so strong as that which is produced after immunization with A blood corpuscles, but that is probably due to the fact that immunization with sheep red corpuscles only gives rise

Table 4.

maturation of A₁ blood corpuscles.

[illegible]

... of 561 blood corpuscles.

Hemolysis of S61 blood corpuscles.		1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096
Before immun.:	R49 abs. with O ~ S61:	80	60	40	20	0	100	80	60	20
After	" " " " ~ "	100	100	100	100	100	100	100	100	100
Before immun.:	R51 abs. with O ~ S61:	80	80	40	20	0	0	0	0	0
After	" " " " ~ "	40	20	0	0	0	0	0	0	0

of the complement with alcoholic extract of guinea pig kidney.

Fixation of the complement with alcoholic extract of	1/16	1/32	1/64	1/128	1/256	1/512
Before immun.: R49 abs. with O ~ GP extr.: 10	10	40	40	100	100	100
After " " " " " ~ "	0	0	0	40	60	100
Before immun.: R51 abs. with O ~ GP extr.: 60	60	80	100	100		
After " " " " " ~ "	40	60	80	100		

Table 4 shows the determination of A agglutinin, sheep neurophysin sera before and during immunization with A1 blood corpuscles.

to production of A antibody opposite the A antigen of these corpuscles, and not opposite the total A antigen such as it is contained in human blood corpuscles.

A peculiar phenomenon, which was also reported by *T. Andersen*, was observed in these experiments, namely, that whether sheep red corpuscles with much or little A antigen were used for the immunizations, there was only produced A antibody opposite the A antigen contained in the blood corpuscles of all sheep but never opposite that antigen which is contained in the blood corpuscles of some sheep only.

For the sake of comparison these 20 animals were moreover immunized with human blood corpuscles of type A, and the result was perfect agreement, for all those animals which, after immunization with sheep red corpuscles, produced antibody opposite the A antigen, did so, too, after immunization with A blood corpuscles, whereas none of the other animals was able to do so.

Conclusion.

These experiments show, on the whole that, even though it is not warrantable simply to distinguish between those rabbits which are, and those which are not, capable to produce antibody (by far the majority of the animals producing a certain small quantity), there is nevertheless a pronounced difference in the capability of rabbits to develop antibodies. Thus from these experiments it is evident that the rabbits are divided into two approximately equal groups.

The animals of the one group produce, besides species-specific antibody, only a small quantity of A agglutinin and no sheep hemolysin.

The animals of the other group produce, besides species-specific antibody, strong A agglutinin and strong sheep hemolysin.

The difference between the two groups is most distinctly evidenced by the hemolysins corresponding to the A antigen which is found in some sheep only, as these hemolysins are

not present naturally in the body but are only formed in response to immunization. In the animals of the first named group *no* antibody of this kind is formed, whereas *all* the animals of the second group produce such hemolysin with a high titer (2^9 — 2^{16}).

Summary.

In order to ascertain the capability of rabbits to produce antibodies after immunization with human blood corpuscles of type A, 40 rabbits were immunized with A_1 blood corpuscles, and 14 rabbits, with A_2 blood corpuscles.

The antibody formation proved to be the same whether blood corpuscles of type A_1 or of type A_2 were used.

According to their capability to produce antibodies the rabbits could be divided into two well characterized and almost equal groups. The animals of the one group produced very little, a few of them no agglutinin whatever opposite A blood corpuscles, and neither sheep hemolysin nor complement-fixing antibody opposite extract of guinea pig kidney. On the other hand, all the animals of the other group produced strong A agglutinin, sheep hemolysin, and complement-fixing antibody opposite extract of guinea pig kidney.

By immunization with sheep red corpuscles quite the same distribution of the rabbits was found, for antibodies opposite the A antigen of the sheep red corpuscles were only produced by those animals which were able to do so also after immunization with human blood corpuscles of type A.

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ÜBER MEHRERE NEUE SALMONELLA-TYPEN.

Von F. Kauffmann.

(Eingegangen bei der Redaktion am 29. November 1940).

In dieser Arbeit soll über mehrere neue Salmonella-Typen berichtet werden, und zwar zunächst über verschiedene Typen der Salmonella B-Gruppe.

I. Typen der Salmonella B-Gruppe.

1. *Salmonella saint-paul*, Edwards und Bruner.

Antigenstruktur I.IV.V.XII... e,h \longleftrightarrow 1,2,3...

Es handelt sich hierbei um einen von P. R. Edwards und D. W. Bruner bestimmten Typus, der aus einer von Pomeroy erhaltenen Kultur, die aus einer Geflügel-Leber stammte, isoliert wurde (zusammen mit *S. anatum* und *S. litchfield*). Ich konnte die von Edwards und Bruner angegebene Antigenformel bestätigen.

2. *Salmonella zagreb*, Kauffmann.

Antigenstruktur IV.V.XII... e,h \longleftrightarrow 1,2...

Es handelt sich hierbei um 6 von N. Černozubov, Zagreb, unter der Diagnose »S. reading« erhaltene Kulturen, die jedoch von diesem Typus serologisch verschieden sind. Wie man aus den Antigenformeln von *S. saint-paul* und *S. zagreb* ersieht, beruht der serologische Unterschied zwischen diesen beiden Typen auf dem verschiedenen Bau der unspezifischen Phase, zu deren Charakterisierung wir das Zeichen »3«, das

*) Diese Arbeit wurde mit finanzieller Unterstützung des Commonwealth Fund, New York, ausgeführt.

früher zwecks Vereinfachung aus dem Antigenchema gestrichen war, wieder gebrauchen. Die Anwesenheit des I-Antigens bei *S. saint-paul* — im Gegensatz zu *S. zagreb* — ist wahrscheinlich ohne Bedeutung, da man bei *S. saint-paul* mit dem Vorkommen von Stämmen ohne dieses Antigen rechnen muss, während auch umgekehrt *S. zagreb*-Kulturen mit dem I-Antigen auftreten könnten.

3. *Salmonella kaposvar*, Rauss.

Antigenstruktur IV.V.XII... e,(h) \longleftrightarrow 1,5...

Der von *K. Rauss* gefundene und bestimmte Typ unterscheidet sich von *S. reading* vor allem durch die spezifische Phase, die sich — ebenso wie die spezifische Phase von *S. onderstepoort* — von allen anderen e,h-Phasen unterscheidet. Der Anwesenheit des V-Antigens können wir keine diagnostische Bedeutung beimessen, da wahrscheinlich auch *S. kaposvar*-Stämme ohne das V-Antigen vorkommen.

Es muss damit gerechnet werden, dass in den obigen Formeln nicht alle Antigendifferenzen zwischen *S. saint-paul*, *S. zagreb*, *S. kaposvar* und *S. reading* angegeben sind, da nähere Untersuchungen noch nicht durchgeführt wurden. Hier sollte nur darauf hingewiesen werden, dass bestimmte *Salmonella*-Typen mit *S. reading* nahe verwandt sind.

4. *Salmonella californica*, Edwards, Bruner und Hinshaw.

Antigenstruktur IV.XII... g,m,t... — .

Im Jahre 1940 von *P. R. Edwards*, *D. W. Bruner* und *W. R. Hinshaw* aus Puten isoliert und als neuer Typ beschrieben. Ich konnte die obige Antigenformel bestätigen.

5. *Salmonella arechavaleta*, Hormaeche und Peluffo.

Antigenstruktur IV.[V].XII... a \longleftrightarrow 1,7...

Ein von *E. Hormaeche* und *C. A. Peluffo*, Montevideo, gefundener Typus, der von mir noch nicht untersucht wurde. Die Publikation von *Hormaeche* und *Peluffo* befindet sich im Druck.

6. *Salmonella altendorf*, Hohn.

Antigenstruktur IV.XII... c \longleftrightarrow 1,7...

Ein von J. Hohn 1940 bestimmter Typus, dessen Antigenformel von mir bestätigt wurde. Isoliert bei akuter Gastroenteritis.

Über die Antigenbeziehungen dieser 6 neuen Typen der *Salmonella* B-Gruppe zu bereits bekannten Typen gibt die Tabelle 1 Auskunft.

Tabelle 1.

Typ	O-Antigen	H-Antigen	
		1. Phase	2. Phase
<i>S. saint-paul</i>	I.IV.V.XII...	e,h	1,2,3...
<i>S. zagreb</i>	IV.V.XII...	e,h	1,2...
<i>S. kaposvar</i>	IV.V.XII...	e,(h)	1,5...
<i>S. reading</i>	IV.XII...	e,h	1,5...
<i>S. derby</i>	[I].IV.XII...	f,g...	—
<i>S. essen</i>	IV.XII...	g,m...	—
<i>S. budapest</i>	[I].IV.XII...	g,t...	—
<i>S. californica</i>	IV.XII...	g,m,t...	—
<i>S. arechavaleta</i>	IV[V].XII...	a	1,7...
<i>S. altendorf</i>	IV.XII...	c	1,7...
<i>S. abortus ovis</i>	IV.XII...	c	1,6...

II. Typen der *Salmonella* C-Gruppe.1. *Salmonella hartford*, Edwards und Bruner.

Antigenstruktur VI₁.VI₂.VII... y \longleftrightarrow e,n,x...

Ein von P. R. Edwards und D. W. Bruner bestimmter Typ, der nach meinen Untersuchungen ammonschwach ist.

2. *Salmonella montevideo* 2, Kauffmann.

Antigenstruktur VI₁.VII... g,m,s... —.

Bei der kulturellen Untersuchung eines hier in Kopenhagen (Diagnose-Abteilung des Instituts) isolierten Monte-

video-Stammes fiel mir auf, dass dieser im Gegensatz zu allen anderen 22 untersuchten Montevideo-Kulturen Inosit und l-Weinsäure prompt spaltete. Es lag daher nahe, auch an eine serologische Abweichung zu denken, die in der Tat durch die nähere Analyse des O-Antigens zu Tage trat. Während alle übrigen Kulturen von *S. montevideo* die O-Antigene VI₁.VI₂.VII enthalten, fehlt dem frisch isolierten, hiesigen Stamme das VI₂-Antigen.

3. *Salmonella oregon*, Edwards und Bruner.

Antigenstruktur VI₁.VIII... d \longleftrightarrow 1,2,3...

Bei 2 von *P. R. Edwards* und *D. W. Bruner* erhaltenen Kulturen Nr. 103 und 1712 handelt es sich in Bestätigung von *Edwards* und *Bruner* um Kulturen, die *S. muenchen* = VI.VIII. d \longleftrightarrow 1,2... nahe stehen, aber den 3-Faktor in der unspezifischen Phase enthalten. Zwischen den beiden Stämmen 103 und 1712 bestehen kulturelle Unterschiede, die aus der Tabelle 7 hervorgehen.

4. *Salmonella manhattan*, Edwards und Bruner.

Antigenstruktur VI₁.VIII... d \longleftrightarrow 1,5...

Auch diese von *P. R. Edwards* und *D. W. Bruner* erhaltene Kultur hat — in Bestätigung der Angaben von *Edwards* und *Bruner* — eine von *S. muenchen* abweichende unspezifische Phase; doch sind nähere Antigenanalysen noch nicht durchgeführt, sodass mit weiteren, serologischen Unterschieden, speziell innerhalb des komplex gebauten d-Antigens oder O-Antigens zu rechnen ist.

5. *Salmonella litchfield*, Edwards und Bruner.

Antigenstruktur VI₁.VIII... l,v \longleftrightarrow 1,2,3...

Es handelt sich hierbei um einen 1940 von *P. R. Edwards* und *D. W. Bruner* bestimmten Typus, der aus einer von *Pomeroy* erhaltenen Kultur, die aus einer Geflügel-Leber stammte, isoliert wurde (zusammen mit *S. anatum* und *S. saint-paul*). Ein zweiter Stamm von *S. litchfield* wurde von *Edwards* und *Bruner* unter Kulturen festgestellt, die sie 1932

von G. Cooper erhalten hatten, und der aus einer menschlichen Nahrungsmittelvergiftung stammte. Ich konnte die Antigenformel bestätigen.

6. *Salmonella duesseldorf*, Hohn.

Antigenstruktur VI₁.VIII... z₄,z₂₄.

Dieser neue von J. Hohn erhaltene Typ — mit der Angabe, dass er das VI.VIII-Antigen und ein nicht bestimmbares H-Antigen besitzt — ist deswegen von Interesse, da er im H-Antigen serologische Beziehungen zu *S. cerro* aufweist, sodass dieser Typus nun in das *Kauffmann-White-Schema* eingeordnet werden kann. Da ich gleichzeitig einen anderen neuen Typus »*S. arizona*« untersuchte, der ebenfalls zu *S. cerro* H-Antigenbeziehungen hat, so sollen die gegenseitigen Antigenbeziehungen dieser 3 Typen gemeinsam weiter unten bei *S. arizona* besprochen werden.

Über die Antigenbeziehungen dieser 6 neuen Typen der *Salmonella* C-Gruppe zu bereits bekannten Typen gibt die Tabelle 2 Auskunft.

Tabelle 2.

Typ	O-Antigen	H-Antigen	
		1. Phase	2. Phase
<i>S. bareilly</i>	VI ₁ .VI ₂ .VII	y	1,5...
<i>S. hartford</i>	»	y	e,n,x...
<i>S. mikawashima</i>	»	y	e,n,z ₁₅ ...
<i>S. montevideo</i> 1	VI ₁ .VI ₂ .VII...	g,m,s...	—
<i>S.</i> » 2	VI ₁ .VII...	g,m,s...	—
<i>S. muenchen</i>	VI ₁ .VIII	d	1,2...
<i>S. oregon</i>	»	d	1,2,3...
<i>S. manhattan</i>	»	d	1,5...
<i>S. litchfield</i>	VI ₁ .VIII	l,v	1,2,3...
<i>S. duesseldorf</i>	»	z ₄ ,z ₂₄	—

III. Typen der Salmonella D-Gruppe.

1. *Salmonella onarimon*, *Kisida*.

Antigenstruktur I.IX.XII... $b \longleftrightarrow 1,2 \dots$

Ich erhielt diese Kultur von *S. Kisida* und konnte auf Grund einer orientierenden Untersuchung die Angaben von *Kisida* bestätigen.

Dieser Stamm, der das H-Antigen von *S. paratyphi* B besitzt, verhält sich kulturell wie *S. paratyphi* B: Er vergärt nicht d-Tartrat und bildet typischen Schleimwall. *H. Anzai* und *H. Tsurumi* bestätigten ebenfalls die Angaben von *S. Kisida* und wiesen *S. onarimon* bei einem typhusähnlichen Krankheitsbilde zusammen mit Paratyphenterie-Bacillen nach.

2. *Salmonella goettingen*, *Hohn*.

Antigenstruktur IX.XII... $1, v \longleftrightarrow e, n, z_{15} \dots$

Es handelt sich hierbei um einen von *J. Hohn* bestimmten, diphasischen Typus mit α - β -Phasenwechsel, dessen Antigenstruktur IX. 1... $e, n \dots$ von *Hohn* im Prinzip richtig erkannt, jedoch nicht vollständig geklärt war. Die genaue Formel IX.XII... $1, v \longleftrightarrow e, n, z_{15}, z_{17} \dots$ wurde von mir festgestellt.

In der Tabelle 3 sind die Antigenbeziehungen dieser beiden neuen Typen zu bereits bekannten Typen der D-Gruppe dargestellt.

Tabelle 3.

Typ	O-Antigen	H-Antigen	
		1. Phase	2. Phase
<i>S. sendai</i>	[I].IX.XII...	a	1,5...
<i>S. onarimon</i>	I.IX.XII...	b	1,2...
<i>S. dar es salaam</i>	I.IX.XII...	1,w	$e, n \dots$
<i>S. goettingen</i>	IX.XII...	1,v	$e, n, z_{15} \dots$
<i>S. panama</i>	I.IX.XII...	1,v	1,5...

IV. Typen der *Salmonella* E-Gruppe.

1. *Salmonella vejle*, Harhoff.

Antigenstruktur III.X.XXVI. e,h \longleftrightarrow 1,2,3...

Von E. Møller, Kopenhagen, 1940 bei akuter Gastroenteritis isoliert und bestimmt; von N. Harhoff beschrieben.

2. *Salmonella meleagridis*, Edwards und Bruner.

Antigenstruktur III.X.XXVI. e,h \longleftrightarrow 1,w...

Dieser Typus wurde 1940 von P. R. Edwards und D. W. Bruner bestimmt, und zwar an einer Kultur, die aus einer Pute gezüchtet war und weiterhin bei anderem Geflügel sowie einem Falle von menschlicher Gastroenteritis gefunden wurde. Eine zweite Kultur wurde mir später von J. Hohn übersandt, mit der Angabe, dass hier ein neuer Typ vorlag, dessen Antigenformel ungefähr richtig angegeben war.

Beim Vergleich dieser beiden Kulturen ergab es sich, dass sie im Bau des O-Antigens nicht völlig übereinstimmten, da das O-Antigen der originalen Kultur von Edwards vom III.X.XXVI-Antigen etwas verschieden war und sich hierin wie *S. nyborg*, *S. zanzibar* und *S. lexington* verhielt.

Kürzlich erhielt ich 3 Kulturen von *S. meleagridis*, die M. Tesdal aus deutschen Soldaten in Norwegen isoliert und bestimmt hatte.

3. *Salmonella lexington*, Edwards, Bruner und Rubin.

Antigenstruktur III.X.XXVI. z₁₀ \longleftrightarrow 1,5...

Von P. R. Edwards, D. W. Bruner und H. L. Rubin 1940 als neuer Typ beschrieben; aus den Mesenterialdrüsen gesunder Schweine isoliert. Ich fand, dass dieser Typ im O-Antigen gering von anderen III.X.XXVI-Kulturen abweicht und einen Sonderfaktor enthält.

Auf Grund einer persönlichen Mitteilung ist dieser Typ gleichzeitig und unabhängig von Edwards, Bruner und Rubin durch M. Erber in Batavia festgestellt worden, da er dieselbe Antigenformel angab. Die von Erber angekündigten Kulturen sind jedoch noch nicht in Kopenhagen eingetroffen, sodass

die Identität dieser Kulturen mit der amerikanischen Kultur nicht festgestellt werden konnte. Erber schlug vor, diesen Typ »*S. batavia*« zu nennen.

4. *Salmonella illinois*, Edwards und Bruner.

Antigenstruktur III.XV... $z_{10} \longleftrightarrow 1,5...$

Im Jahre 1940 auf Grund einer persönlichen Mitteilung von P. R. Edwards aus Puten und aus einem Schwein isoliert. Die Publikation von P. R. Edwards und D. W. Bruner befindet sich im Druck.*)

In der folgenden Tabelle 4 sind die Antigenbeziehungen dieser 4 Typen zu *S. anatum* dargestellt.

Tabelle 4.

Typ	O-Antigen	H-Antigen	
		1. Phase	2. Phase
<i>S. anatum</i>	III.X.XXVI.	e,h	1,6...
<i>S. vejle</i>	„	e,h	1,2,3...
<i>S. meleagridis</i>	„	e,h	1,w
<i>S. lexington</i>	„	z_{10}	1,5...
<i>S. illinois</i>	III.XV...	z_{10}	1,5...

V. Typen weiterer Gruppen.

1. *Salmonella borbeck*, Hohn und Herrmann.

Antigenstruktur XIII.XXII. $1,v \longleftrightarrow 1,6...$

1940 von J. Hohn und W. Herrmann als neuer Typus beschrieben; aus den Faeces eines an Typhus erkrankten Kindes isoliert. Eine Nachprüfung konnte nicht erfolgen, da nach Mitteilungen von Hohn diese Kultur abgestorben ist.

2. *Salmonella wichita*, Schiff und Strauss.

Antigenstruktur I.XIII.XXIII. d... —.

1939 von F. Schiff und L. Strauss als neuer Typus beschrieben, und zwar an 3 Kulturen, die B. McKinlay bei

*) Das O-Antigen von *S. illinois* ist von dem III.XV-Antigen verschieden und enthält einen Sonderfaktor.

einem Enteritisausbruch unter Neugeborenen in Wichita, Kansas, isoliert hatte. Ich konnte die Antigenformel bestätigen.

3. *Salmonella havana*, Schiff und Saphra.

Antigenstruktur I.XIII.XXIII. f, g. . . —.

Auf Grund einer persönlichen Mitteilung von I. Saphra, New York, 1940 als neuer Typus bestimmt; von mir noch nicht untersucht. Die Isolierung dieses Typus erfolgte 1937 durch A. Curbelo, der diese Keime bei einem Ausbruch von Meningitis, der 21 Neugeborene des »Maternity Hospital« in Havana betraf, aus Lumbalflüssigkeit, Blut und Faeces isolierte. Sämtliche Kinder starben. Die Seren von 3 Angestellten des Hospitals agglutinierten *S. havana* bis 1:150; doch wurden keine *Salmonella*-Bacillen aus den Faeces dieser Personen isoliert.

4. *Salmonella cerro*, Hormaeche, Peluffo und Salsamendi.

Antigenstruktur XVIII. z_4, z_{23}, z_{25} .

1938 von E. Hormaeche, C. A. Peluffo und R. Salsamendi als neuer Typus beschrieben, aus den Mesenterialdrüsen gesunder Schweine und aus dem Menschen isoliert. Auf Grund des kulturellen Verhaltens wurde der Stamm als wahrscheinlicher Angehöriger der *Salmonella*-Gruppe angesehen, jedoch nicht in das *Kauffmann-White-Schema* eingefügt, weil keine Antigenbeziehungen zur *Salmonella*-Gruppe nachweisbar waren. Ich gab die Formel mit XVIII. z_4 an.

1940 konnte ich fast gleichzeitig 2 verschiedene neue Typen: *S. duesseldorf* und *S. arizona* untersuchen, die mit *S. cerro* gemeinsame H-Antigene besitzen. Da *S. duesseldorf* die VI.VIII-Antigene enthält, so steht der Einordnung von *S. cerro* und *S. arizona* in das *Kauffmann-White-Schema* nichts mehr im Wege. Die nähere H-Antigenanalyse wird bei *S. arizona* erörtert.

5. *Salmonella urbana*, Edwards und Bruner.

Antigenstruktur XXX. $b \longleftrightarrow e, n, x. . .$

1940 von P. R. Edwards und D. W. Bruner als neuer

Typus beschrieben (im Druck). Ich konnte die Angaben von Edwards und Bruner bestätigen und stellte fest, dass *S. urbana* ein auf *S. hvittingfoss* übergreifendes O-Antigen besitzt, das jedoch schwächer als das Hauptantigen XXX entwickelt ist.

6. *Salmonella arizona*, Kauffmann.

Antigenstruktur XXXIII. z_4, z_{23}, z_{26} .

Im Jahre 1939 beschrieben M. E. Caldwell und D. L. Ryerson in ihrer Arbeit »Salmonellosis in certain reptiles« eine Kultur unter dem Namen »*Salmonella dar es salaam*, variety from Arizona«, die für »horned lizards (*Phrynosoma solare*), Gila monsters (*Heloderma suspectum*) und chuckawalla (*Sauromalus ater*)« sowie für Meerschweinchen und Kaninchen pathogen war. Da diese Kultur sich in kultureller Hinsicht ähnlich wie *S. dar es salaam* verhielt — sie verflüssigte auch langsam Gelatine, war aber in Dulcitol negativ — und in Seren von *S. dar es salaam*, *S. reading* und *S. newport* agglutinierte, so wurde sie ohne nähere Antigenanalyse als zu *S. dar es salaam* gehörend betrachtet.

Ich erhielt diese Kultur von M. E. Caldwell und bezeichne sie im folgenden als »*S. arizona*«, da es sich hierbei um einen neuen serologischen Typus handelt, der unter Berücksichtigung des Kauffmann-White-Schema keine serologischen Beziehungen zu *S. dar es salaam* besitzt. Die kulturellen Unterschiede zwischen *S. dar es salaam* und *S. arizona* gehen aus der Tabelle 7 hervor, die zeigt, dass *S. arizona* langsam (nach ca. 2 Wochen) und unregelmässig Lactose spaltet — im Gegensatz zu *S. dar es salaam*. Hiermit ist also ein weiterer lactosespaltender *Salmonella*-Typ bekannt, nachdem ich zuerst bei einem Stamme von *S. anatum* die Lactosespaltung nachwies und in einer vorhergehenden Arbeit über verschiedene »*Salmonella coli*«-Typen, die Lactose spalten, berichtete.

In serologischer Hinsicht verhielt sich der Stamm »*Arizona*« schwach rauh und gab keine stabile Suspension in Alcohol. Dagegen waren die Aufschwemmung lebender Bacillen in 0,9 % NaCl-Lösung und die gekochte Bouillonkultur

stabil. Die lebende und gekochte Kultur agglutinierten in verschiedenen Salmonella-Seren, und zwar vor allem in solchen, die Rauh-Antikörper enthielten, z. B. in den beiden H-Seren c und d der Internationalen Salmonella-Centrale, die mit Rauh-Kulturen hergestellt waren, und in den beiden Typhus Vi-Seren, die mit den Kulturen »6 S« und »Vi I« hergestellt waren. Da die Arizona-Kultur weder die H-Antigene c und d noch das Vi-Antigen enthielt, so müssen diese Reaktionen als Rauh-Agglutinationen betrachtet werden, zumal keine gemeinsamen glatten O-Antigene vorhanden waren.

Es geht aus diesem Beispiele hervor, dass es sehr bedenklich ist, diagnostische H- und Vi-Seren mit Rauh-Formen herzustellen, da es hierdurch leicht zu Fehldiagnosen kommen kann. Da wir nämlich über die zweifellos sehr komplexe Natur der Rauh-Antigene und -Antikörper noch sehr ungenügend orientiert sind, so können wir nicht durch Absorption mit bestimmten Rauh-Kulturen alle Rauh-Antikörper, z. B. aus Vi-Seren, mit Sicherheit entfernen.

Ich möchte daher auf Grund dieser praktischen Erfahrungen, die sich nicht nur auf *S. arizona*, sondern auch auf andere Kulturen erstrecken, empfehlen, alle diagnostischen Seren nur mit Glattformen herzustellen.

Die serologische Analyse von *S. arizona* wurde mit Hilfe von H-Seren, O-Seren und von Seren, die durch Immunisierung mit lebenden Bakterien hergestellt waren, durchgeführt. Bei der Immunisierung mit lebenden Kulturen erwies sich *S. arizona* als hoch pathogen für Kaninchen, da trotz vorsichtiger Dosierung mehrere Kaninchen starben. Ein Vi-Antikörper konnte in diesen Seren nicht nachgewiesen werden.

S. arizona besitzt ein neues O-Antigen XXXIII, das zum IX-Antigen keine Beziehungen hat. Das H-Antigen ist komplex gebaut und hat zu *S. cerro* und *S. duesseldorf* nahe Beziehungen, die in den folgenden Formeln ausgedrückt sind:

$$\begin{array}{ll} \text{S. duesseldorf} & = \text{VI. VIII. } z_4, z_{24} \\ \text{S. cerro} & = \text{XVIII. } z_4, z_{23}, z_{25} \\ \text{S. arizona} & = \text{XXXIII. } z_4, z_{23}, z_{26} \end{array}$$

Ein Phasenwechsel konnte bei diesen 3 Typen bisher nicht nachgewiesen werden; doch wurden keine Versuche unternommen, durch Züchtung auf H-Immunserum enthaltenden Nährböden einen Phasenwechsel herbeizuführen.

Die H-Antigenstruktur dieser 3 Typen wurde durch Absorptionsversuche und Reagensglas-Agglutinationen festgestellt, die in der Tabelle 5 wiedergegeben sind.

Tabelle 5.
Ergebnisse der H-Reagensglas-Agglutination.
H-Immunseren.

Agglutination mit Kultur	S. duesseldorf			S. cerro			S. arizona		
	nicht absorbiert	absorbiert mit		nicht absorbiert	absorbiert mit		nicht absorbiert	absorbiert mit	
		S. cerro	S. arizona		S. duesseldorf	S. arizona		S. duesseldorf	S. cerro
S. duesseldorf	6400	1600	3200	800	< 50	< 50	1600	< 50	< 50
S. cerro	800	< 50	< 50	3200	1600	800	3200	800	< 50
S. arizona	800	< 50	< 50	3200	800	< 50	12800	6400	3200

Über die Antigenbeziehungen dieser 6 neuen Typen zu bereits bekannten Typen gibt die Tabelle 6 Auskunft.

Tabelle 6.

Typ	O-Antigen	H-Antigen	
		1. Phase	2. Phase
S. poona	XIII.XXII.	z...	1,6...
S. borbeck	XIII.XXII.	1,v	1,6...
S. worthington	I.XIII.XXIII.	1,w	z...
S. wichita	I.XIII.XXIII.	d...	—
S. havana	I.XIII.XXIII.	f,g...	—
S. cerro	XVIII.	z ₄ , z ₂₃ , z ₂₅	—
S. urbana	XXX.	b	e,n,x...
S. arizona	XXXIII.	z ₄ , z ₂₃ , z ₂₆	—

Über das kulturelle Verhalten der oben genannten Typen gibt die Tabelle 7 Auskunft.

Tabelle 7.

	S. saint-paul	S. zagreb	S. ka-pos-var	S. reading	S. reading Z.*	S. california	S. alten-dorf
Zahl der Stämme	1	6	1	2	1	1	1
Adonit, Lactose, Saccharose, Salicin	— ³⁰	— ³⁰	— ³⁰	— ³⁰	— ³⁰	— ³⁰	— ³⁰
Indol	—	—	—	—	—	—	—
Arabinose, Dextrin, Dextrose, Dulcit, Mannit, Maltose, Sorbit, Trehalose, Xylose, H ₂ S	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹
Inosit	+ ¹	— ³⁰	— ³⁰	+ ¹	— ³⁰	+ ¹	+ ¹
Rhamnose	+ ¹	+ ¹	+ ¹	×	+ ¹	+ ¹	+ ¹
Gelatine	— ⁶⁰	— ⁶⁰	— ⁶⁰	— ⁶⁰	— ⁶⁰	— ⁶⁰	— ⁶⁰
Stern Glycerin	+++ ²	+++ ²	— ⁸	— ^{od.} +++ ²	+++ ²	+++ ²	+++ ²
d-Tartrat, Na-Citrat, Mukat	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹
l-Tartrat i- „	+ ¹ + ³	+ ¹ + ³⁻⁴	+ ⁶⁻⁷ — ¹⁴	+ ³⁻⁸ — ¹⁴	×	+ ¹ — ¹⁴	+ ¹ — ¹⁴
Simmons Agar mit Arabinose, Dextrose, Dulcit und Na-Citrat	+	+	+	+	+	+	+
Simmons Agar mit Rhamnose	+	+	+	—	+	+	+

*) Ob der mit »Z« bezeichnete Reading-Stamm aus Zanzibar mit S. reading serologisch identisch ist, kann noch nicht gesagt werden.

Tabelle 7 Fortsetzung.

	S. hart- ford	S. mon- tevi- deo 1	S. mon- tevi- deo 2	S. muen- chen	S. ore- gon 103	S. ore- gon 1712	S. man- hat- tan	S. litch- field	S. dues- sel- dorf
Zahl der Stämme	1	22	1	11	1	1	1	2	2
Adonit, Lactose, Sac- charose, Salicin	— ₃₀	— ₃₀	— ₃₀	— ₃₀	— ₃₀	— ₃₀	— ₃₀	— ₃₀	— ₃₀
Indol	—	—	—	—	—	—	—	—	—
Arabinose, Dextrin, Dextrose, Dulcit, Man- nit, Maltose, Rham- nose, Sorbit, Treha- lose, Xylose, H ₂ S	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹
Inosit	— ₃₀	— ₃₀	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	— ₃₀	— ₃₀
Gelatine	— ₆₀	— ₆₀	— ₆₀	— ₆₀	— ₆₀	— ₆₀	— ₆₀	— ₆₀	— ₆₀
Stern Glycerin	+++ ³	+++ ²⁻³	+++ ²	+++ ²⁻³	— ⁸	+++ ²	+++ ³	+++ ²	+++ ²
d-Tartrat	+ ²⁻³	+ ¹	+ ¹	+ ¹	+ ¹	×	+ ¹	+ ¹	+ ¹
l- »	+ ¹	×	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	×
i- »	+ ³	— ¹⁴	— ¹⁴	+ ¹⁻⁴	+ ³⁻⁴	+ ²⁻³	+ ³	+ ³⁻⁵	— ¹⁴
Na-Citrat	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ²
Mukat	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ²⁻³
Simmons Agar mit Arabinose, Dextrose, Dulcit, Rhamnose, Na- Citrat	—	+	+	+	+	+	+	+	+

Tabelle 7 Fortsetzung.

	S. ona- rimon	S. goet- tingen	S. vejle	S. mele- agri- dis	S. lex- ing- ton	S. wi- chita	S. cerro	S. urba- na	S. arizo- na
Zahl der Stämme	1	1	1	5	1	2	2	1	1
Adonit, Saccharose, Salicin	— ³⁰	— ³⁰	— ³⁰	— ³⁰	— ³⁰	— ³⁰	— ³⁰	— ³⁰	— ³⁰
Lactose	— ³⁰	— ³⁰	— ³⁰	— ³⁰	— ³⁰	— ³⁰	— ³⁰	— ³⁰	×
Indol	—	—	—	—	—	—	—	—	—
Arabinose, Dextrin, Dextrose, Mannit, Mal- tose, Rhamnose, Sor- bit, Trehalose, Xylose, H ₂ S	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹
Dulcit	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	— ³⁰
Inosit	+ ¹	— ³⁰	+ ¹	+ ¹	+ ¹	— ³⁰	— ³⁰	— ³⁰	— ³⁰
Gelatine	— ⁶⁰	— ⁶⁰	— ⁶⁰	— ⁶⁰	— ⁶⁰	— ⁶⁰	— ⁶⁰	— ⁶⁰	—+
Stern Glycerin	++ ⁸	++ ⁶	++ ²	++ ²	++ ²	++ ²	++ ²	++ ⁵	++ ²
d-Tartrat	— ¹⁴	+ ¹	+ ¹	+ ¹	+ ²	+ ¹	+ ¹	+ ¹	— ¹⁴
l- »	+ ¹	+ ⁵⁻⁷	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ⁵⁻⁶	— ¹⁴
i- »	+ ²	— ¹⁴	+ ³	+ ³⁻⁴	— ¹⁴	+ ³⁻⁴	— ¹⁴	— ¹⁴	— ¹⁴
Na-Citrat	+ ¹	+ ²	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ²
Mukat	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹	+ ¹
Simmons Agar mit Arabinose, Dextrose, Na-Citrat	+	+	+	+	+	+	+	+	+
Simmons Agar mit Dulcit	+	+	+	+	+	+	+	+	—
Simmons Agar mit Rhamnose	+	+	+	+	+	+	+	—	+

Zeichenerklärung:

+¹ = positiv nach 1 Tage; +³⁻⁴ = positiv nach 3—4 Tagen;
 —³⁰ = negativ nach 30 Tagen; × = spät und unregelmässig posi-
 tiv, oft negativ. Bei Stern Glycerin: ++² = lila nach 2 Tagen.
 Bei Gelatine: —+ = langsam verflüssigend.

Sämtliche Kulturen bildeten Gas aus Dextrose und Mannit.
 S. onarimon bildete typischen Schleimwall.

Zusammenfassung.

Es werden serologische und kulturelle Untersuchungen an mehreren neuen Salmonella-Typen mitgeteilt.

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DESTRUCTION OF GROUP ANTIGENS BY ENZYMES.

By *Grethe Hartmann and Jul. Hartmann.*
(Received for publication December, 2nd 1940).

1^o Introduction.

In 1931 *Schiff* (6—7) discovered that a regular, spontaneous destruction of the group antigen present in saliva and feces took place at a temperature of 37° C. So far, however, the question has not been settled whether this destruction is due, in the main or even exclusively, to bacterial enzymes or to an enzyme produced by the organism itself. Two conceptions have been suggested. In the first instance *Schiff* (8) and his co-workers have held the view that the phenomenon is due to organic enzymes. The following observations speak in favour of this conception.

- 1) It is possible to obtain saliva with a considerable amount of cells and antigen destroying enzymes »Blutgruppenferment«, by energetic mastication directly after rinsing the mouth.
- 2) The »Blutgruppenferment« can be shown in the mucous membrane of the stomach from persons of normal acidity although no trace of enzyme is found in the gastric juice of such persons.
- 3) Saliva provoked by irritation of the parasympathicus (pilocarpine) contains very little enzyme as against saliva provoked by irritation of the sympathicus (adrenalin). Opposed to *Schiff's* views is the conception maintained

particularly by *Witebsky* and by *Sievers*, according to which the destruction is due, at any rate chiefly, to bacterial enzymes. The following arguments may be put forward in favour of this interpretation.

- 1) The »Blutgruppenferment« cannot be identified with any known digestive enzymes. (11—9).
- 2) The destruction of the blood group antigen is accelerated if certain nutritive substances (*Witte's* peptone or bouillon) are added to the mixture of antigens and enzymes. (12—10).
- 3) Transfers through a number of identical nutritive substances is possible. However, a greater quantity of the sample in question is required than is normally necessary for the transfer of bacteria. (12—10).
- 4) Further it may be noted that a number of bacteria are able to destroy the group antigens. This holds good of the *Welsh-Fränkel* bacillus, the *Lamb* bacillus of dysentery, the bacillus ovitoxicus (5—3) and certain bacteria dissociating carbohydrates viz. myxococcus *Morgan-Thayesen* and saccharobacterium ovale (*Landsteiner* 4).

The question as to the part played by bacterial enzymes in the spontaneous destruction of the group antigens could obviously not be settled on the basis of the earlier investigations. It was, however, evident that in the investigations on the effect of the enzymes of the organs proper on the antigens the bacterial enzymes were likely to falsify the results. For instance it would seem probable that this source of error has played an essential part in *Schiff's* investigations on the enzymes in the mucous membrane of the stomach. *W. Henle* (2) has examined whether a difference could be traced in the effect on the destruction of group antigens of the enzymes in saliva from the so-called secreters and non-secreters. The result was negative. This does not, however, prove that there is no difference in the content of enzymes, for instance of the salivary glands, seeing that such a difference might be masked by bacterial enzymes in the non-

sterilized salivas. The question about a difference in the content of enzymes within the two types will be considered in the following.

2° *The Method used for the Determination of the Antigen Concentration.*

In the examinations below salivas from secreters of group A_1 and B were used as test material for the effect of the group destroying enzymes. For the measurement of the antigen concentration in the salivas the agglutination inhibition method was employed in the following form: Two series of small test tubes were used in the determination of the antigen content of each sample. Into the first tube of each series 0.1 cc was introduced of the original antigen solution, (concentration 1), in the second 0.1 cc of a concentration $\frac{1}{2}$, in the third 0.1 cc of a concentration $\frac{1}{4} = \frac{1}{2^2}$, in the fourth 0.1 cc of a concentration $\frac{1}{8} = \frac{1}{2^3}$ etc. Thus in the tubes no. n 0.1 cc of an antigen solution of concentration $\frac{1}{2^{n-1}}$ was inserted. Hereafter 0.1 cc of a serum anti-A was entered into all the tubes of one of the two series while 0.1 cc of a serum anti-B was added to the content of all the tubes of the other series. Thus, after this operation, the antigen concentrations in the tubes of each of the two series were $\frac{1}{2^1} - \frac{1}{2^2} - \frac{1}{2^3} - \frac{1}{2^4} - \dots - \frac{1}{2^n}$. In the following experiments the same isosera (anti-A = Ulla $\frac{1}{16}$; anti-B = Claus $\frac{1}{16}$) were employed throughout the investigations. These two sera proved rather constant as to the strength. After the introduction of the serum the test tubes were kept at about 20° C. for an hour when washed blood corpuscles of group A_1 and B were added, the A_1 blood corpuscles to the test tubes with serum iso-anti-A, the B blood corpuscles to the test tubes with serum iso-anti-B. After two hours the tubes were shaken. In one of the two series agglutination is seen to take place in all the tubes while in other agglutination occurs only in the tubes following a certain number n (provided that saliva of group A_1 or B only are considered). The number n is taken as a measure of the antigen concentration in the original antigen solution (the saliva) and is termed the titer reading for this solution. It may be noted that n is the power of 2 in the expression for the antigen concentration in the tube no. n after the addition of serum.

3° *Blood-Group-destroying Enzymes in the Submaxillary Glands.*

We now proceed to consider some experiments on the content of blood-group-destroying enzymes in organs. In the experiment an initially sterile material was absolutely essential in order to avoid interference with bacterial enzymes originally found in the material. For this reason the gastric mucosa were left out of consideration and the submaxillary gland from corpses without infections (death caused by accidents) was chosen. The following dissection technique was employed.

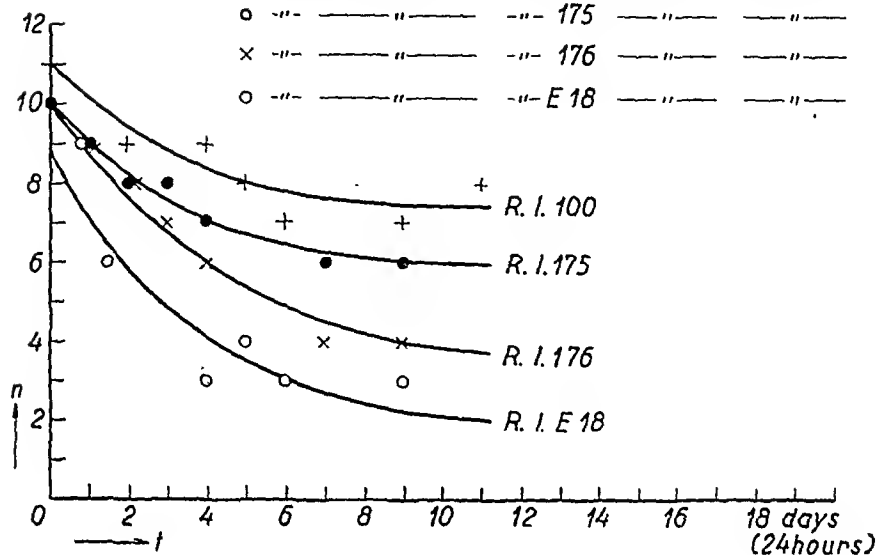
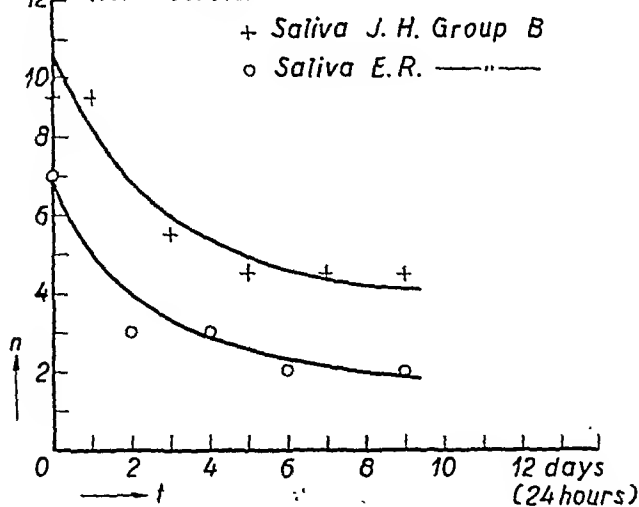
After application of iodine to the skin a cutaneous cut is made with a sterile knife parallelly to the mandible and down along the front of the throat. The skin is turned aside and with another sterile knife and a tweezer the gland is removed. The gland is cut into small pieces and two grams of the mass is introduced into a test tube containing 2 cc of boiled and sterile saliva and kept at 37° C. As a check on sterility, especially with regard to anaërobies which bacteria — according to previous investigations (*Schiff*² — *Kostuch*³ — *Hartmann*¹) — would seem to be particularly rich on the enzyme in question, a small portion of the content of the test tube is added to cystein-agar likewise kept at 37° C. At intervals of 24 to 48 hours the antigen concentration in the saliva is measured by the agglutination inhibition method. For the sake of comparison, pure boiled saliva kept at 37° C. without organic mass is simultaneously examined in the same way. Figs. 1 a—d show the results of the main tests. (Saliva with submaxillary gland).

Those samples only are considered which preserved complete sterility, as tested by cystein-agar, for at least 6 days. Two samples of the submaxillary gland from non-secreters of group A₁ were tested, one of the samples, fig. 1 b, in connection with two, the other, fig. 1 c, in connection with three different salivas of group B.

In addition four samples of the submaxillary gland from

Saliva J. H. Group B

+ Gl. Submaxillaris R.I. 100 Secreter. Group A₁
 o " " " " " 175 " " " " "
 x " " " " " 176 " " " " "
 o " " " " " E 18 " " " " "

Gl. Submaxillaris. R. I. 179 Group A₁
Non Secreter

Gl. Submaxillaris. R. I. 102 Group A₁
Non Secreter

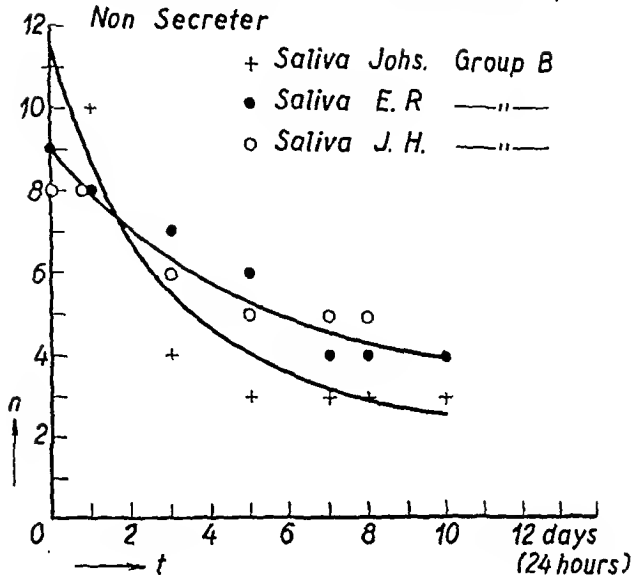


Fig. 1c.

Gl. Submaxillaris E. 26 Group O.

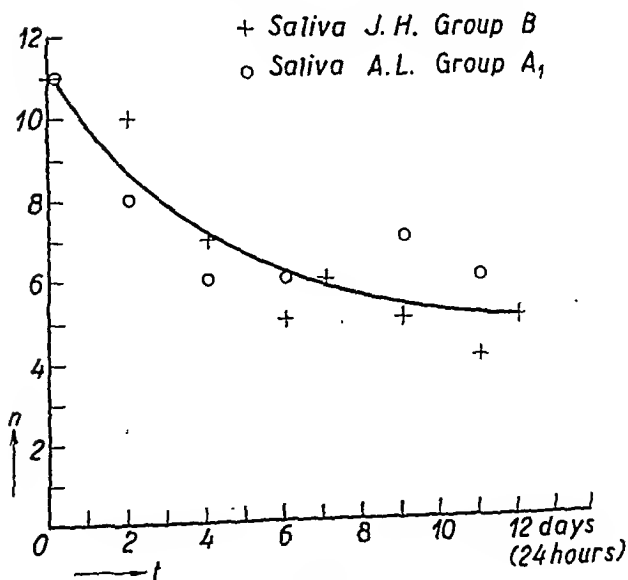


Fig. 1d.

secreters of group A_1 were tested with a certain saliva of group B, fig. 1 a, and finally one single submaxillary gland of group O was tested with the latter saliva and further with a saliva of group A_1 , fig. 1 d. The figures show smoothed-out curves representing the decrease of the antigen content in the course of time under the influence of the submaxillary gland. The decrease is much the same whether the submaxillary gland comes from a secreter or a non-secreter. Actually greater variations are found between the separate curves in the case of secreters than between the average curves for secreters and non-secreters. It appears from the curves that there is no basis for the assumption of a difference between the two types, secreters and non-secreters, as to the content of a blood-group-destroying enzyme in the submaxillary gland.

The comparatively regular shape of the curves representing the reduction in the course of time of the antigen content in salivas under the influence of the organic enzymes and, further, the great resemblance which these curves bear to each other, suggest a simple process behind the observed changes. We will try to form an idea of this process. We shall imagine the reduction of the antigen content to be due to the destructive action of enzymes from the organ introduced into the saliva. We shall assume that initially the antigen has a concentration C_0 and the enzymes a concentration Z_0 . Further it is assumed that the enzymes vanish in the course of time according to a formula typical of such a reduction, viz.

$$(1) \quad Z = Z_0 e^{-\alpha t}$$

Figs. 1a—d.

Curves for the destruction of Group Antigen in Saliva under the Influence of Enzymes from the Submaxillary Gland: a) Saliva, Group B, with Submaxillaries from Secreters of Group A_1 . b) Salivas, Group B, with Submaxillaries from a Non-Secreter of Group A_1 . c) 3 Salivas, Group B, with Submaxillaries from a Non-Secreter of Group A_1 . d) Salivas, Groups A_1 and B, with Submaxillaries from a Person of Group O.

where α is a constant and e the base of the natural logarithms. Finally the assumption is made that the rate at which the antigen concentration C is reduced is at any moment proportional to the product of the values of C and Z obtaining at that moment. This assumption is expressed analytically in the equation

$$(2) \quad \frac{dC}{dt} = kCZ.$$

Introducing Z from (1) into (2) this equation may be written

$$(3) \quad \frac{dC}{C} = -kZ_0 e^{-\alpha t} dt.$$

Integration of (3) gives

$$(4) \quad \ln \frac{C}{C_0} = -\frac{kZ_0}{\alpha} (e^{-\alpha t} - 1)$$

or what amounts to the same thing

$$(5) \quad \frac{C}{C_0} = e^{-\frac{kZ_0}{\alpha} (e^{-\alpha t} - 1)}.$$

Translating now to titer readings n by means of the relations

$$(6) \quad \frac{C}{C_0} = \frac{2^n}{2^{n_0}} = 2^{n-n_0}$$

we may write (5) as

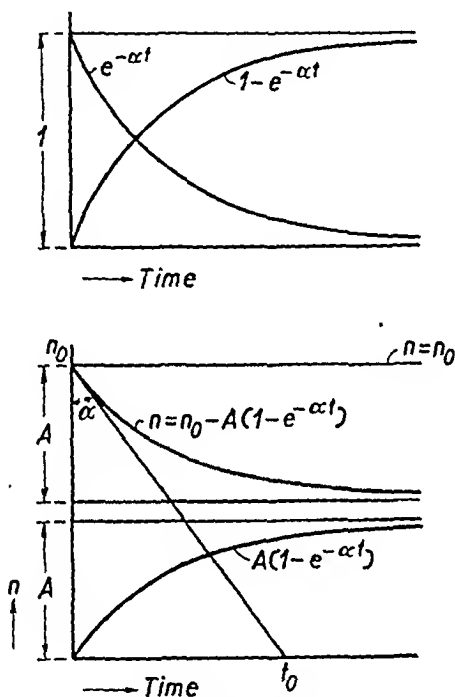
$$(7) \quad 2^{n-n_0} = e^{-\frac{kZ_0}{\alpha} (e^{-\alpha t} - 1)}$$

which, again, may be written

$$(7a) \quad n = n_0 - \frac{kZ_0}{\ln 2} \cdot (1 - e^{-\alpha t}) = n_0 - A (1 - e^{-\alpha t}).$$

Fig. 2 is, now, to explain how the curve representing the formula (7_a) is built up and how a formula of the character given by (7_a) may be fitted to a given experimental curve. In the uppermost system of coordinates the curve $e^{-\alpha t}$ is drawn. It starts with the value 1 and drops to zero in the course of infinite time. Further in the same system the curve

$1 - e^{-\alpha t}$ is represented. The way in which it is constructed is obvious. Hereafter we shall imagine all the ordinates of the latter curve to be multiplied by $A = \frac{kZ_0}{\alpha l_e 2}$. From the new ordinates the lower curve in the lower system of coordinates



Figs. 2a-b.

Nature of the Curve used for representing the Destruction of Group Antigens by organic Enzymes.

is drawn. It thus represents $A(1 - e^{-\alpha t})$, i. e. the second term on the right hand side of formula (7_n). Finally the ordinates of the latter curve are subtracted from the line $n = n_0$. The curve produced in this way is that represented by (7a), thus the curve for the decay of the antigen concentration expressed in exponential titers n . Now in the actual case the experimental curve for the decay is given and we have to examine whether it can be represented by a curve

of the type $n = n_0 - A(1 - e^{-\alpha t})$. The problem is then to determine the three constants n_0 , A and α from the experimental curve. When this has been done the curve represented by the formula may readily be calculated and compared with the experimental curve. The determinations of the constants may be performed in the following way. In the first instance the values of n_0 to be used in the formula is appropriately chosen as the initial ordinate of the experimental curve. This means that we shall let the theoretical curve start at the same titer reading as the experimental curve. Then, if we are able to estimate the ordinate of the horizontal asymptote to which the experimental curve approaches we have immediately A , for the asymptote should, as seen from fig. 2, be the distance A below the line $n = n_0$. It remains to find α . This is done by determining the slope of the experimental curve at its uppermost point $n = n_0$. The slope of the curve represented by (7_a) is

$$\frac{dn}{dt} = -A\alpha e^{-\alpha t}.$$

Hence, at the point $t = 0$, $\frac{dn}{dt} = -A\alpha$. The slope of the experimental curve is found by drawing the tangent to the curve at its uppermost point. If the tangent cuts the axis of abscissa in the point t_0 then, fig. 2, the slope is $\frac{n_0}{t_0}$. Identifying this slope with $-A\alpha$ we find α , seeing that A was already known. Hereafter all three constants of the formula (7_a) are determined. It will now be gathered that they have been chosen in such a way that the theoretical curve 1) passes through the same starting point n_0 as the experimental, 2) has the same tangent in this point as the latter curve, 3) has the same horizontal asymptote as this curve. In order to ascertain how closely the theoretical curve fits the experimental curve a suitable number of values are given to t and the corresponding values of n are calculated from (7_a) after the introduction of the values for n_0 , A and

Submaxillaris. Secreters

$$\times n = 10.4 - 5.7[1 - e^{-0.269t}]$$

o Experiment

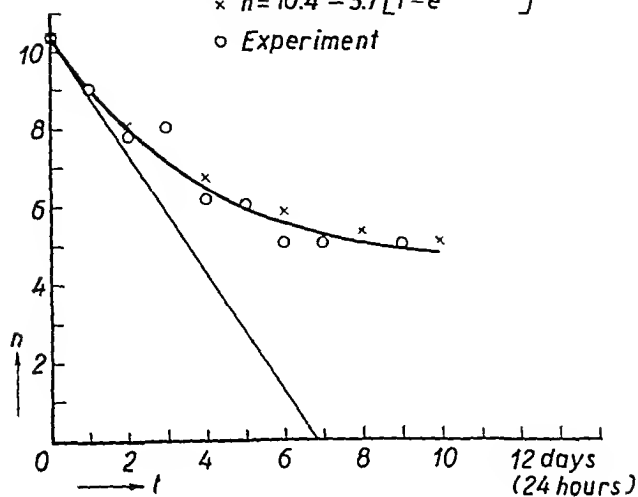


Fig. 3a.

Submaxillaris. Non-Secreters.

$$\times n = 9.0 - 5.7[1 - e^{-0.336t}]$$

o Experiment

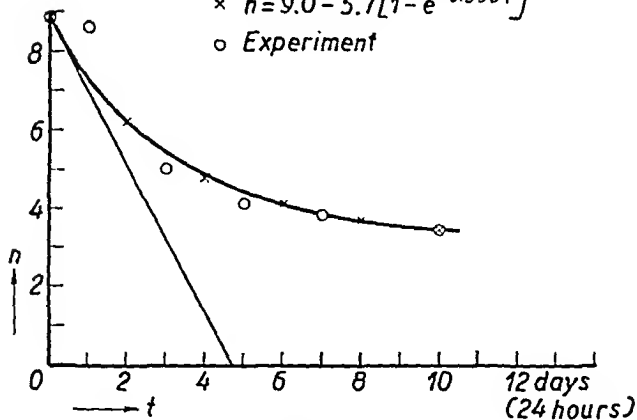


Fig. 3b.

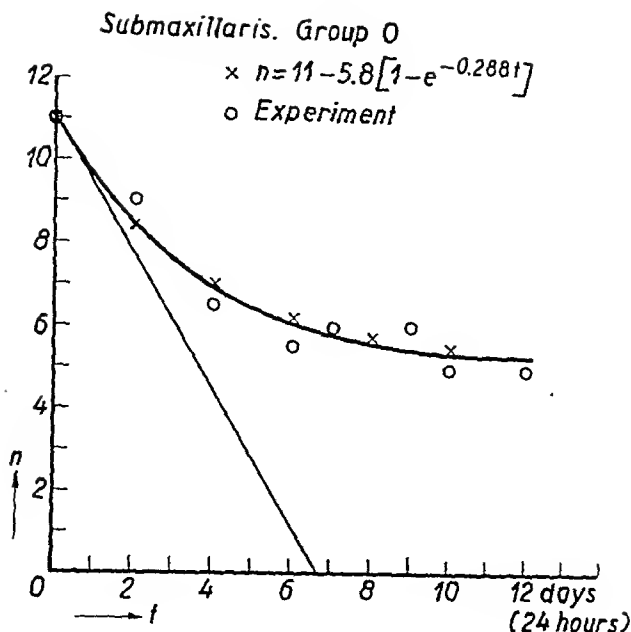


Fig. 3c.

Figs. 3a—c.

Average Curves showing the Destruction of Group Antigens in Saliva under the Influence of: a) Submaxillaries from Secreters, b) Submaxillaries from Non-Secreters and c) Submaxillaris from a Person of Group O.

α found as described above. The values of n are plotted against t in the same system of coordinates in which the experimental curve is drawn. If the latter curve can be represented by the formula the theoretical points should coincide or nearly coincide with the experimental curve. In order to try this average curves were derived from the direct observations. These curves are shown in figs. 3 a—c together with the corresponding theoretical curves.

It is seen that the curves drawn on the basis of observations can actually, with almost astonishing exactness, be pictured by formula (7_a) which represents our theory of the process of antigen destruction by organic enzymes. This fact would

seem to render it practically certain that the conceptions upon which this theory is built up are substantially true.

It was stated that in the tests considered in figs. 1 a—d those samples only were utilized which kept sterile through-

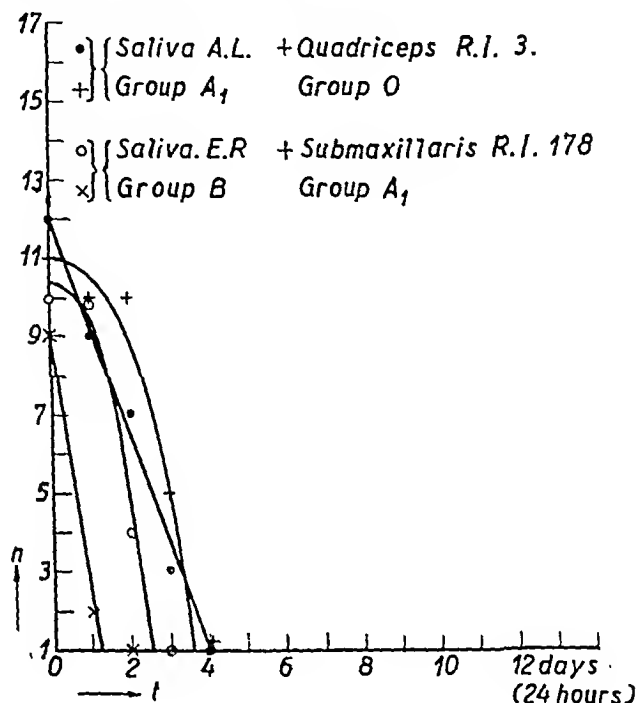


Fig. 4.

Destruction of the Group Antigen in Saliva when infected by Bacteria of Putrefaction.

out the period of the experiment in question. Complete sterility was hardly to be reckoned with even with very careful treatment. After the lapse of about a week a minute growth of bacteria could as a rule be traced, though it had no perceptible influence on the further course of the process. In some of the tests, however, a pronounced development of bacteria of putrefaction was seen in the course of the first 24—48 hours. The presence of these bacteria completely changed the conditions in as much as a rapid and complete destruc-

tion of the group antigens took place in such cases. This destruction is illustrated in fig. 4. The great part played in the blood group destruction by bacteria as compared with that of the organic enzymes proper is obvious.

4° Blood-Group-destroying Enzymes in the Quadriceps Muscle.

Finally a few tests were made with a non-glandular organ in order to find out whether the antigen-destroying enzymes are distributed throughout the organism or are found in those organs only which as the submaxillary gland contain an ample amount of group antigens. A muscle (the quadriceps) was chosen because this organ has neither any secretion nor a high antigen content. Two samples, one from a person of group O and another from a person of group A_1 were examined as to their antigen destroying effect, with

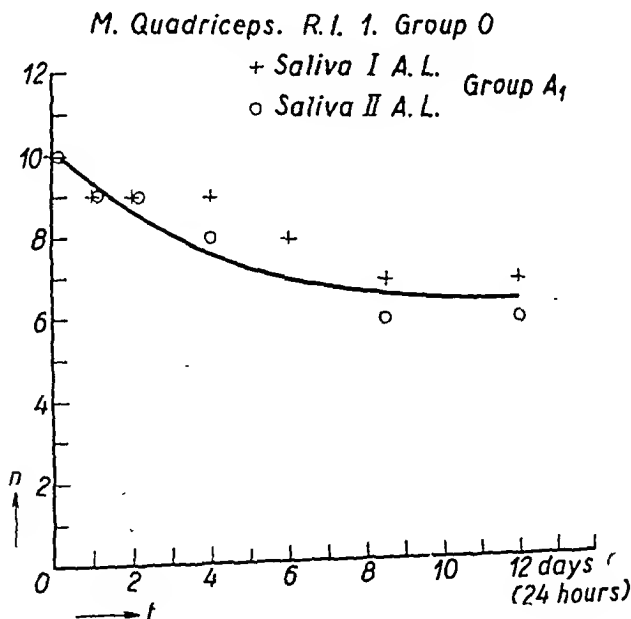


Fig. 5a.

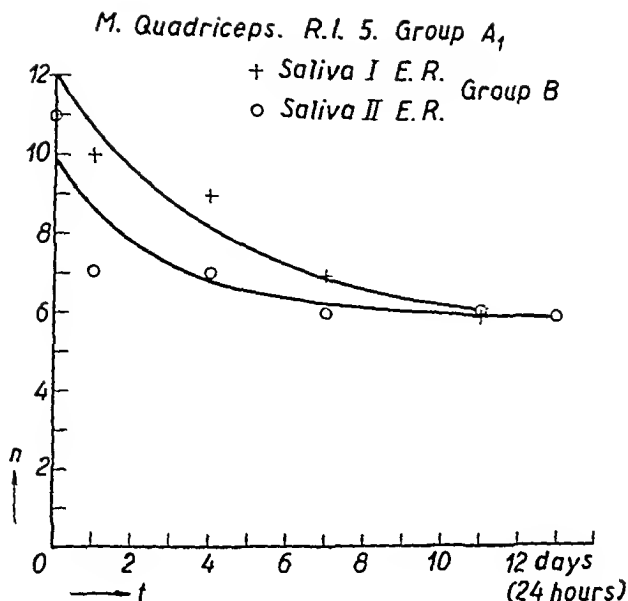


Fig. 5b.

Figs. 5a—b.

Curves for the Destruction of Group Antigen in Saliva under the Influence of Enzymes in the Quadriceps Muscle: a) Saliva, Group A₁ with Quadriceps, Group O. b) Saliva, Group B with Quadriceps, Group A₁.

salivas from two individuals, one of group A₁ and the other of group B. As will appear from figs. 5 a—b a similar, though perhaps a somewhat slighter effect than that found with the submaxillary gland was established.

In fig. 6 the average of two of the curves is shown. The curve may be represented by an expression of the same form as was used to picture figs. 3 a—c. Points calculated from the expression in question are marked out in the figure.

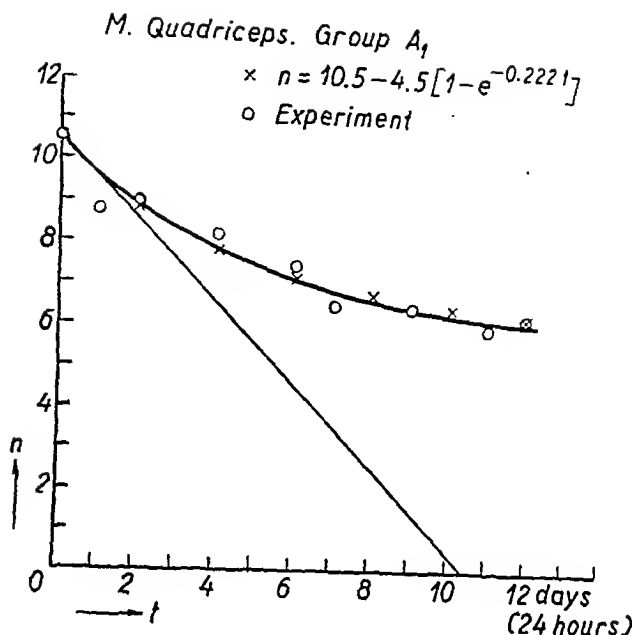


Fig. 6.

Average Curve showing the Destruction of Group Antigen in Saliva under the Influence of Enzymes from the Quadriceps Muscle.

5° Investigations on the spontaneous Destruction of the Group Antigens in Saliva.

It was found of interest to supplement the investigations now recorded with some observations on the spontaneous destruction of the group antigen in saliva. If recently secreted saliva which is not made sterile by boiling or by any other means is kept at 37° C. all the group antigen is, as a rule, destroyed in less than 24 hours. At the same time a rich culture of bacteria will develop, as established by microscopic tests or even by the smell. If, on the other hand, the saliva is boiled, it may retain its original content of antigen for more than a month at least. It would seem that the destruction has some close relation to the development of bacteria, seeing that samples which keep relatively free from micro-organisms exhibit a slow destruction (complete break-

Table I.
Spontaneous Destruction of Blood Group Antigen in Saliva
at 37° Celcius.

		Hours					
Sample of Saliva	Blood Group	0	24	48	72	96	120
Henry	A ₁	11		11		5	1
Lise	A ₁	10		10		1	0
Karl	B	10		9		0	
Erik	A ₁	10	10	6	0		
	B	5	5	4	0		
Robert	B	9	9	1	0		
Alice	A ₁	3	3	1			
Sven	A ₁	9	9	6	5		1
Yrsa	A ₁	9	8	1	0		
Inge	A ₁	11	2	0			
Aage	A ₁	11	10	1			
Johan	A ₁	8	8	3	0		
Markus	A ₁	11	11	5	1	0	
Ole, boiled	A ₁	10		10			10

down in the course of up to a week) while such as develop plenty of bacteria show a more rapid decay.

Instances of the normal destruction are given in Tab. I and in figs. 7 a—d. It will be noted that the square of the time has been chosen as abscissa in the figures, while the ordinate is the ratio of the titer reading n in question and the initial titer reading n_0 . With the said abscissa the smoothed-out curves are straight lines radiating from point 1 on the axis of ordinates. The only exception from the straight

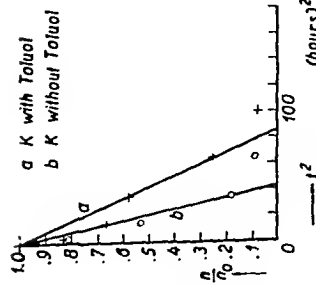


Fig. 7a.

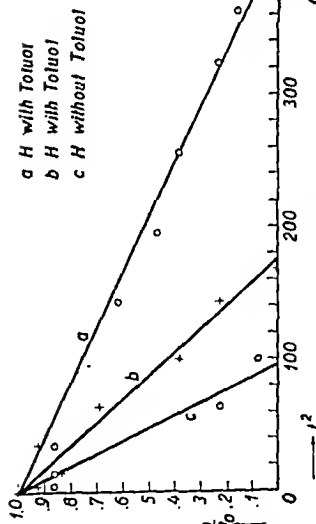


Fig. 7b.

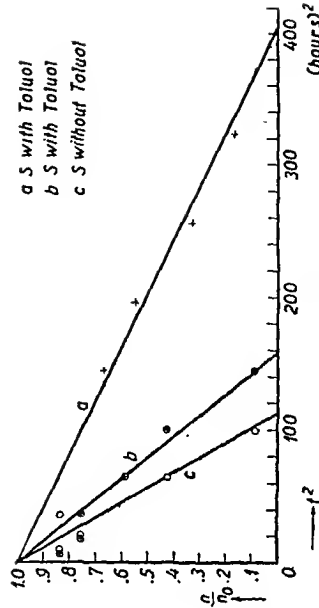


Fig. 7c.

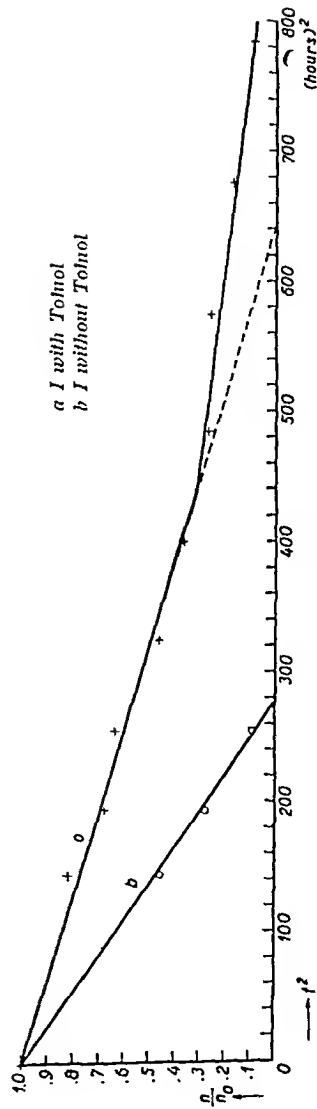


Fig. 7d.

Figs. 7a—d.

Spontaneous Destruction of Group Antigen in Saliva: Abscissa: Square of the Time, Ordinate: Titer Reading measured with Initial Titer Reading as Unit.

line was found in the case of a very slow decay. An example is seen in fig. 7_d curve *a*, corresponding to a very »pure« saliva treated with toluol, which has always the effect of retarding the decay. This fact is illustrated in the figures from which other information also may be gained. Thus it is seen that the rate of decay of saliva samples from different days may vary greatly. In figs. 7_b and 7_c curves *b* and *c* correspond to the same saliva sample, while curve *a* corresponds to saliva from another day.

The curves picturing the spontaneous decay of the group antigen in saliva are still simpler than those representing the effect of the organic enzymes, at least when drawn in the way indicated above. They undoubtedly reflect a process of equally simple structure. An attempt will now be made to form a picture of this process in the shape of an analytic theory. The conceptions on which the proposed theory are based are 1) that the destruction of the antigen is due to enzymes produced by bacteria in the saliva; 2) that the content or concentration of bacteria *B* increases in the course of time, from a minute amount to a stationary concentration B_{∞} according to the law:

$$(1) \quad B = B_{\infty} (1 - e^{-\beta t})$$

where *e* is the base of the natural logarithms and β a constant; 3) that the rate at which the antigen vanishes is at any moment proportional both to the antigen concentration *C* and to the bacteria concentration *B* prevalent at that moment. This conception is expressed by the equation:

$$(2) \quad \frac{dC}{dt} = -k_1 \cdot BC.$$

If the expression (1) for *B* is introduced into (2) this equation may be written:

$$(3) \quad \frac{dC}{C} = -k_1 B_{\infty} (1 - e^{-\beta t}) dt.$$

The integral of (3) is:

$$(4) \quad l_e \left(\frac{C}{C_0} \right) = -k_1 B_{\infty} \left(t + \frac{1}{\beta} (e^{-\beta t} - 1) \right)$$

where C_0 is the initial concentration and l_e the logarithm with the base e . With sufficiently small values of t we may replace $e^{-\beta t}$ by the three first terms of its series development, i. e. we may write:

$$(5) \quad e^{-\beta t} = 1 - \beta t + \frac{1}{2} (\beta t)^2.$$

If this expression is introduced into (4) we get

$$(4a) \quad l_e \left(\frac{C}{C_0} \right) = -k_1 B_\infty \frac{\beta t^2}{2}$$

or what is the same thing:

$$(4b) \quad \frac{C}{C_0} = e^{-k_1 B_\infty \frac{\beta}{2} \cdot t^2} = e^{-rt^2}.$$

The assumption underlying the expressions (5), (4a) and (4b) means that the increase of B may, throughout the process of destruction of the antigen, be pictured by the formula:

$$(6) \quad B = B_\infty \beta t \left(1 - \frac{1}{2} \beta t \right)$$

or that the said process covers only the first part of the growth of the bacteria colony. It may be that it covers a greater part of the growth when more terms of the series development should be employed. If two more terms are used formula (4b) is replaced by:

$$(4c) \quad \frac{C}{C_0} = e^{-rt^2} \left(1 - \frac{1}{3} \beta t + \frac{1}{12} (\beta t)^2 \right).$$

If it covers the complete growth of the bacteria colony to the final steady state then it follows from (4) that

$$(4d) \quad \frac{C}{C_0} = e^{-k_1 B_\infty \left(t + \frac{1}{\beta} (e^{-\beta t} - 1) \right)}.$$

Returning, now, to the simplest case represented by (4b) and replacing the C coordinate with the titer reading n by means of the relation:

$$(7) \quad \frac{C}{C_0} = 2^{n - n_0}$$

we get instead of (4b):

$$(8) \quad 2^n - n_0 = e^{-\gamma t^2} \quad \text{or}$$

$$(8a) \quad n - n_0 = -\frac{\gamma}{\ln 2} \cdot t^2 \quad \text{or again}$$

$$(8b) \quad n = n_0 \left(1 - \frac{\gamma}{n_0 \ln 2} \cdot t^2\right) \quad \text{or}$$

$$(8c) \quad \frac{n}{n_0} = 1 - \epsilon t^2 \quad \text{where } \epsilon \text{ is a constant.}$$

Hence, if $\frac{n}{n_0}$ is plotted against t^2 the curve found should be a straight sloping line starting from $\frac{n}{n_0} = 1$. So it is seen that the theory predicts just the sort of curve which is actually found.

6° Conclusions.

Our experiments on the spontaneous destruction of group antigen in saliva would seem to throw light on the question whether this destruction is due solely to bacterial enzymes or partially also to enzymes originating in the salivary glands. We have seen that the spontaneous destruction is such as would take place if it were caused by bacteria capable of producing blood-group-destroying enzymes and capable of reproduction in saliva. Similarly, we found that the antigen destruction in sterile saliva under the influence of raw organ could be explained as the effect of a substance which, itself, was destroyed in the course of time, thus a substance behaving as an organic enzyme is generally assumed to behave under the experimental conditions in question. Now, as to the question of the possible participation of organic enzymes in the spontaneous destruction of antigen in saliva, our experiments permit us to say, that, *if a certain amount of organic enzymes is transmitted to the saliva this amount is at any rate so minute that it is not able to change the process of destruction to such an extent as to render it distinguishable from a purely bacterial destruction.* In contra-

distinction it should be noted that an admixture of bacteria is likely to completely mask the process of destruction due to organic enzymes.

It was established that the antigen-destroying enzyme was found not only in the submaxillary gland, but also in an organ like the quadriceps muscle which is relatively poor in group antigens and has no secretion whatever. This observations suggest that the enzyme in question is present, in a more or less pronounced degree, in the whole organism. So, it may perhaps be considered not as an enzyme acting specifically on group antigens but rather as an enzyme capable of destroying polysaccharides in general. Such enzymes are not known to any great extent within the human organism, while they play a great part within the domain of the anaërobies, i. e. the bacteria which are generally in a high degree active in the spontaneous destruction of group antigen in saliva. In this connection attention may be called to *Landsteiner's* (4) observation of the fact that certain bacteria which are able to destroy polysaccharides are also capable of destroying group antigens.

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EXPERIMENTS OF PROVOKING PERNICIOSIFORM ANEMIA IN PIGS BY PYLORECTOMY.

By *Erik Bandier*.

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Introduction.

During recent years numerous attempts have been made to provoke experimental pernicious (perniciosiform) anemia in various species of animals. Very seldom has it been possible to provoke anemia with even the slightest resemblance to pernicious anemia, and these results have always failed to be reproducible.

As experimental animals besides the usual laboratory animals (mice, rats, guinea pigs) were particularly used dogs, and finally pigs, too. Thus, *Bence* (1933 and 1936), *Maison & Ivy* (1934), *Goodman et al.* (1935), and *Petri et al.* (1937 and 1938) have subjected pigs to total gastrectomy for that purpose. However, the anemia entailed by that operation was hypochromic in all the cases.

I. Previous Investigations.

Prompted by *Meulengracht's* reports (1934 and 1935), according to which the pyloric part of the stomach of pigs is of the greatest effectiveness in the treatment of pernicious anemia, *Waterman et al.* (1937) subjected four 3 month old Yorkshire pigs to pylorotomy ad modum Billroth II. Three

of the animals survived the operation and, in the course of 3—4 months, developed pronounced anemia, slightly hyperchromic as compared to the values found prior to the operation. One of the animals 4 and 5 months after the operation received intramuscular injections of large doses of liver extract (pernaemon and pernaemon forte). It is reported that the diameter of the erythrocytes did not undergo any change either after the operation or after the injection of liver extract. On the other hand it is reported that, after injection of »pernaemon forte«, a strong reticulocyte reaction was observed besides a pronounced increase of the hemoglobin per cent as well as of the number of red corpuscles. This does not appear distinctly from the experimental reports, however.

II. Own Investigations.

In order to ascertain whether the removal of the pyloric part of the stomach of pigs really entailed hyperchromic anemia responding to liver therapy (in contradistinction to total gastrectomy which, as was previously mentioned, was followed by hypochromic microcytic anemia) I performed the following experiments.

A. Material, operation, experimental arrangement.

The experimental animals were eight 5 month old »Chinese« (black) pigs*) of the same litter. The reason why they were preferred to ordinary Danish pigs was that they are much smaller and, perhaps, somewhat hardier than the Danish pigs.

Three of the animals, which served as controls, were not operated upon. The other 5 animals were subjected to re-

*) The pigs were supplied and placed at the disposal of the Institute through the courtesy of »Medicinalco«, Ltd., Copenhagen.

section*) ad modum Billroth I of the pyloric part of the stomach (i. e. the duodenal stump is anastomosed end-to-end with the remainder of the stomach) and not, as in Waterman et al.'s case (1937), ad modum Billroth II (closure of the duodenal stump and formation of gastrojejunostomy). Distally, the resection was made in or just distally to the pyloric ring. The resected pieces were submitted to microscopy which, proximally (orally), revealed in all of them a narrow border of fundus mucosa, except quite upward near the small curvature, where the pyloric mucosa by a narrow part continues into the cardia mucosa which is microscopically of a fairly similar structure. All the 5 animals survived the operation and seemed subsequently to feel perfectly well. During the first 5 days post operationem they received milk and water, afterwards, oatmeal-porridge, and after the lapse of 8 days they received their usual food consisting of rye-bread, potato-peel, oatmeal-porridge, crushed red maize, rough-ground barley groats, sweet-milk, besides a daily addition of 10 gr. of cod-liver oil and about 15 gr. of the following salt mixture: Sodium chloride in the proportion of 1 to 1.5 of calcium carbonate and of 2.5 of phosphate calcium.

Before I proceed to the description of the results I shall account for the details of the technic employed for the addition of 10 gr. of cod-liver oil and about 15 gr. of the following salt mixture: Sodium chloride in the proportion of 1 to 1.5 of calcium carbonate and of 2.5 of phosphate calcium.

B. Technic of examining blood samples from pigs.

This technic requires detailed discussion, because the osmotic conditions of pig erythrocytes differ from those of human erythrocytes, whence it is in several respects impossible forthwith to apply

*) Three hours prior to operation the animals received an intramuscular injection of 5 ml. of a 10 % solution of phenobarbital sodium, and during the very operation, a small quantity of ether on an open mask. I am indebted to the analysts A. Jørgensen, A. Rasmussen, and L. Martinsen for valuable assistance in the performance of the operations.

the usual methods of examining human blood to the examination of blood samples from pigs. If a drop of perfectly fresh pig blood is examined under the microscope, all the erythrocytes are seen to be shaped like thorn-apples, and this also holds if heparin has been added to the blood. Also in *Hayem's* and in *Christensen & Warburg's**) solutions as well as in a 0.9 % sodium chloride solution, the pig erythrocytes present thorn-apple shape, and this likewise applies to dilutions of the named solutions, whereas, in *Ringer's* solution and in a 1.3 % sodium oxalate solution, they are round, biconcave, having quite the same appearance as human erythrocytes.

The changes in the technic of blood examination necessitated by the mentioned phenomena are described in the following.

The drawing of blood samples. Venepuncture being fairly difficult, at any rate in small pigs, the blood is drawn after hyperemization (rubbing) of an ear and incision of one of the ear veins. The blood for the different examinations is pipetted directly from the place of incision.

The determination of the hemoglobin content is carried out in the usual manner with the aid of *Hellige's* hemometer.

Erythrocyte count. The erythrocytes are counted in the usual manner in a *Bürker-Türk* counting cell after dilution with *Hayem's* solution, which can very well be used even though the erythrocytes are thorn-apple shaped in it.

The leukocyte count does not either differ from the generally employed method (dilution with a solution of diacetic acid methyl-violet etc.).

Determination of the volume %. To begin with the volume % was determined with the aid of a hematocrit after dilution with *Christensen & Warburg's* solution (cf. above). However, since the erythrocytes were thorn-apple shaped in this solution, the author was afraid that it would not be appropriate for determining the volume % in pig blood. Moreover, the results proved to differ according as the centrifugation was effected at once or after the lapse of 1—2—3 hours. Anyhow, that was unfortunate when the blood samples had to be conveyed a considerable distance before the centrifugation could be done. That is why the volume per cent was determined in »heparin blood«, i. e. without preceding dilution. The volume of erythrocytes was determined as it is in the original plasma (with its Ph, protein and salt concentration), and not under

*) Sodium oxalate 11.300 gr.

Primary potassium phosphate 0.315 gr.

Secondary » » 1.365 gr.

Boiled distilled water to 1000 gr.

standard conditions with the aid of diluted solutions. The procedure was as follows: A 5 % heparin solution is sucked up in the capillary tube of a *C. M. van Allen* hematocrit and blown off again; having ascertained that no drops are left in it the tube is dried in the thermostat. From the place of incision the blood is drawn directly into the tube, up to the mark 100; the heparin adhering to the inner wall of the tube is sufficient to prevent coagulation of the blood. Then centrifugation can be done at any convenient time, a number of examinations having shown that the results are the same whether centrifugation is done at once or after the lapse of up to 4 hours (or even more). The volume per cent is calculated as mean value of from 2 to 4 samples, which seldom present any mutual difference greater than corresponding to about 6–8 per cent of the mean value, and in the majority of cases, even much smaller. For the sake of comparison, the volume % was in 10 cases determined partly in this way and partly with the aid of *Christensen & Warburg's* solution and immediate centrifugation. Good agreement was found in 5 of the cases, whereas, in 4 cases, the values obtained with *Christensen & Warburg's* solution were considerably lower, and in a single case, somewhat higher.

Measuring of the diameter of the erythrocytes. Paying regard, amongst other things, to the osmotic conditions of the erythrocytes, micrometry was performed as follows: On a carefully ungreased groundglass objective a thin smear (objective preparation) is placed very cautiously. Also in the smear the erythrocytes are very apt to become thorn-apple shaped, but some of a nice round shape will always be found to lie freely in the »thin extremity« of the preparation. Having been dried in the air, they are stained with *Jenner's* solution for 2 minutes and, subsequently, washed with phosphate buffer ($\text{Pl} = 6.5\text{--}7$ parts of 1/150 molar primary potassium phosphate and 3 parts of 1/150 molar secondary potassium phosphate). Then they are pressed on filtering paper and dried in the air for 3–5 minutes. Afterwards, staining for 5 minutes with a liquid consisting of 1 part of a 2.5 % eosin solution (water-soluble, yellowish (Grübler)) and 9 parts of phosphate buffer (see above). Finally, washing with buffer and pressing out on filtering paper. Preparations produced in that way will remain unchanged for more than a twelvemonth at any rate. The very micrometry is performed with the help of a microprojection microscope with an enlargement of 1000. In each preparation are counted 100 cells altogether. This is done with a glass scale divided into half millimeters. The method is very accurate; nor is there apparently the same possibility of individual fluctuations with different examiners as in the use of an ocular microscope.

Counting of reticulocytes. Several different methods of counting reticulocytes are known. In the present material, counting of a dry preparation was selected as being the most adequate method. The procedure was as follows: With a pipet, 0.10 cmm. of a 1% brilliant cresyl-blue solution in 1.3% sodium oxalate is placed on a paraffined objective. (In this solution the erythrocytes fail to coagulate and present, moreover, a nice, round, biconcave shape). With the same pipet, 0.10 cmm. of blood is drawn from the ear of the pig and, with the help of a thin, paraffined glass rod, mixed carefully with the brilliant cresyl-blue solution. After 1 minute's standing the mixture is sucked up in the pipet and transferred in two equal portions to a carefully ungreased objective for the »cover-glass preparations«. After drying in the air, 3 minute's fixation in methyl-alcohol and subsequent staining for 10–15 minutes in a mixture of 2 parts of azure-eosin (Giemsa) and 8 parts of phosphate buffer with $\text{Ph} = 6.5$ (see above). The staining liquid is washed off with buffer solution, in which the cells are differentiated for $\frac{1}{2}$ minute and subsequently pressed out on filtering-paper. For the count, a diaphragm with a square opening measuring 3×3 mm. is placed in the ocular. There are counted 1000 erythrocytes altogether. The preparations will remain unchanged for a long time (more than a year).

The above described methods have been employed for $2\frac{1}{2}$ years and proved absolutely satisfactory.

Several authors report great variations in the results of examinations of blood samples of pigs immediately after feeding. That is why the blood is always collected from fasting animals. It is also reported, for example by *v. Falck* (1931), that the hemoglobin content and the proportion of erythrocytes in the circulating blood of pigs present, on the whole, great fluctuations, and it should be of special importance if the experimental animals, before the blood was drawn, had been chased more or less, if they had been restless or not. Greater fluctuations of that kind were not observed in this institute, however. Thus, in a number of cases the animals were killed immediately after the blood samples had been drawn, and examination of the blood flowing out of the jugular gave exactly the same result as the ear blood. Finally, blood specimens drawn successively on a number of days gave fairly identical results (Table 6).

C. Experimental Results.

1. Polyrectomized pigs.

Table 1.

Pig Nr. 1 operated 19/10-1937	19/10	24/11	8/1-38	24/2	26/3*	28/3*	30/3	1/4*	4/4*	6/4	8/4	11/4	19/4	25/4 killed
Hb. %	85	78	76	64	97	75	71	71	69	85	71	75	71	75
Erythrocytes in mill. Diameter of erythro- cytes in μ	7.93	5.11	6.62	5.04	8.04	4.88	4.90	5.12	4.67	5.89	5.32	5.35	4.93	5.00
Volume %	5.9	6.2	6.2	6.2	5.9								5.9	
Reticulocytes ‰	37	32	36	28	43								33	34
Leukoocytes	6	22	7	61	19	11	10	14	16	11	24	13	10	
	17,720	16,800	12,240	19,200	24,600								16,240	

*) Intramuscular injection of 5 ml. of hepsol fortior

Table 2.

Pig Nr. 3 operated 30/10-37	30/10	23/11	8/1-38	24/2	4/4*	6/4*	8/4	11/4*	14/4	19/4	22/4	killed
Hb. %	75	87	84	91	93	96	87	93	93	88		
Erythrocytes in mill. Diameter of erythro- cytes in μ	6.23	6.69	6.90	6.60	7.17	7.45	6.61	6.66	7.00	6.88		
Volume %	6.0	6.1	6.2	6.1	5.9				5.8	5.9		
Reticulocytes ‰	34	38	36	39	40				38	38		
Leukoocytes	12	11	4	4	4	12	4	6	6	12	6	
	18,400	19,440	22,840	22,440	23,840				17,340			

*) Intramuscular injection of 5 ml. of hepsol fortior.

Table 3.

Pig Nr. 5 operated 5/11-1937	5/11	24/11	8/1-38	24/2	4/4*	6/4*	8/4	11/4*	14/4	19/4	24/5 killed
Hb. %	77	80	84	93	97	98	93	95		98	97
Erythrocytes in mill.	6.90	7.50	6.86	7.02	7.57	7.37	7.34	7.06		7.60	7.32
Diameter of erythrocytes in μ	6.1	6.1	6.2	6.0	5.8					5.8	
Volume %	34	36	37	40	41					40	41
Reticulocytes ‰	8	14	2	6	1	5	6	8	4	12	6
Leukocytes	15,840	20,040	20,200	17,640	19,680					19,520	

*) Intramuscular injection of 5 ml. of hepsol fortior

Table 4.

Pig Nr. 2 operated 28/10-1937	28/10	24/11	8/1-38	11/1 died of mechanical ileus (adhesions)
Hb %	78	84	85	
Erythrocytes in mill.	6.00	5.90	6.70	
Diameter of erythrocytes in μ	6.1	6.1	6.1	
Volume %	32	36	35	
Reticulocytes ‰	5	28	4	
Leukocytes	13,000	17,520	20,960	

Table 5.

Pig Nr. 4 operated 2/11-1937	1/11	24/11	14/12	15/12 died of severe chronic changes in both lungs
Hb. %	58	55	42	
Erythrocytes in mill.	5.10	3.52	3.15	
Diameter of erythrocytes in μ	6.0	6.5	6.2	
Volume %	24	22	20	
Reticulocytes ‰	11	22	22	
Leukocytes	14,520	20,000	6,800	

2. Control animals.

Table 6.

Pig Nr. 8, nonoperated control animal	12/11	13/11	14/11	15/11	16/11	17/11	18/11	22/12	19/1-38	24/2 killed
Hb. %	78	79	77	77	79	88	80	89	85	84
Erythrocytes in mill.	6.61	6.65	6.72	6.76	6.70	7.20	6.82	7.22	6.89	6.80
Diameter of erythrocytes in μ	6.1						6.1	6.0	6.1	6.0
Volume %	36	36	35	35	35	36	35	39	38	37
Reticulocytes ‰	14	11	12	10	16	17	13	4	6	8
Leukocytes	17,340	19,600	16,240	19,040	19,880	19,200	20,010	19,600	21,360	

The values noted for the other two nonoperated control animals (pigs Nr. 6 and Nr. 7) were on the very same level. In order to save space, only the initial and terminal values will be recorded for blood specimens collected on the same dates as for the pig Nr. 8: Hemoglobin 77—78 % and 77—91 %; erythrocytes in mill.: 6.20—6.30 and 6.30—7.21; diameter of erythrocytes in μ : 6.0—6.0 and 6.1—6.0; volume %: 35—36 and 35—40; reticulocytes ‰: 26—10 and 17—6; leukocytes: 18,140—18,140 and 18,320—18,000.

3. Conclusion.

As recorded in the Tables 4 and 5, one of the operated animals (Nr. 4) after 6 weeks died of severe chronic changes in both lungs, and another (Nr. 2) after 2½ months died of mechanical ileus due to an intrepitoneal adhesion. The first named animal, which was distinctly anemic already prior to operation, afterwards incurred an exacerbation of the anemia, whereas the other presented no change whatever in the blood picture.

The remaining three pigs (Nrs. 1, 3, and 5) survived the operation in perfect wellbeing until they were killed about 6 months later. Defecation was quite normal; skin and hairy coat as well as growth and increase of length were also in perfect accord with the conditions of the 3 control animals. Nor did the blood picture present any sure deviation from that of the healthy control animals, i. e. signs of perniciosiform anemia failed to appear. Perhaps, the diameter of the erythrocytes presented a dubious, very slight and transitory increase, but, on the whole, the operation had influenced neither Hb. %, number of erythrocytes nor volume %, number of reticulocytes or the Price-Jones curve.

Scarcely 1 month before the animals were killed, i. e. about 5 months after the operation, they were treated with intramuscular injections of large doses of »hepsol fortior«, a very concentrated and strongly anti-anemic liver extract. During this treatment, reticulocyte counts were performed

every day. As is seen from Tables 1, 2 and 3, the injections of liver extract did not affect either the number of reticulocytes or the hemoglobin per cent; nor could that, per se, be expected, since there was no anemia.

To judge from the experiments described in the preceding, the removal of the pyloric part of the stomach in pigs apparently does not entail any changes in the blood picture during the first 6 months at any rate.

Summary.

With a view to provoke perniciosiform anemia, 5 pigs were subjected to pylorectomy a.m. Billroth I.

The details of the technic employed for the examination of blood samples from pigs are accounted for.

To judge from the results of the examinations, the said operation — in contradistinction to the results reported by *Waterman et al.* — does not give rise to distinct changes in the blood picture particularly no changes in the direction of pernicious anemia.

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PHENYLMERCURIC OINTMENTS.

By *Elsa Jensen.*

(Received for publication December 18th 1940).

In two previous papers¹⁾ an account was given of some studies carried out by the writer on the antiseptic properties of phenylmercuric salts. The experiments were made in part with »Merfen«, which is a solution of phenylmercuric borate, partly with various phenylmercuric salts that were placed at my disposal by the Control Laboratory of the Danish Pharmaceutical Association. The tests showed that various phenylmercuric salts have about the same bactericidal effect, and that their bactericidal properties are so marked that they are to be reckoned among the strongest bactericidal substances known so far — an observation that is quite in agreement with the experiments reported by L. A. Weed & E. E. Ecker²⁾, K. E. Birkhaug³⁾, L. P. Garrod⁴⁾, M. Armangue & V. Maestres⁵⁾, O. K. Stark & K. Montgomeri⁶⁾ and N. R. Nye⁷⁾.

As phenylmercuric salts also are relatively non-poisonous, they naturally have been found serviceable for therapeutic use in various infectious diseases amenable to local treatment. Good therapeutic results from their employment in the form of lotions or ointments in the treatment of various affections have been reported by L. H. Biskind⁸⁾, F. W. Hitchins⁹⁾, G. M. Loewe¹⁰⁾, B. M. Stuart¹¹⁾, B. Levine¹²⁾, F. C. Greaves¹³⁾, J. Fariols¹⁴⁾ and B. J. Fog-Møller¹⁵⁾. On employment of watery solutions Fog-Møller found — what is in keeping with my

bacteriological tests — that various phenylmercuric salts have the same therapeutic effect. In his studies Fog-Møller made particular use of phenylmercuric acetate, which acts exactly like merfen.

Three special preparations of phenylmercuric ointment have been put on the market, namely: merfen ointment, merfenil ointment and merfenyl ointment¹⁶). Merfen ointment is a protegin ointment containing 0.4‰ phenylmercuric borate. Merfenil ointment contains 1‰ basic phenylmercuric nitrate in an unknown unguentary substrate; and merfenyl ointment contains 0.66‰ phenylmercuric nitrate in an oxy-cholesterol ointment.

Various phenylmercuric ointment differ in their solubility in water and in different unguentary substrates. Table 1 gives the solubility of the acetate and chloride in water, mineral oil and olive oil as found by J. K. Gjaldbæk & V. Helweg Mikkelsen¹⁷). No report has been published yet on the solubility of phenylmercuric salts in ointment, but it seems reasonable to assume that their solubility in vaseline is about the same as in mineral oil, and that their solubility in unguentary substrates of lipide character is about the same as that in olive oil. At any rate this rule applies to phenol (J. K. Gjaldbæk)¹⁸).

Table 1.

Solubility of	In water	In mineral oil	In olive oil
Phenylmercuric acetate	1:250	1:1600	1:40
Phenylmercuric chloride	1:55000	1:7000	1:500

From Table 1 it is evident that phenylmercuric salts are more soluble in olive oil (corresponding to lard, lanoline, spermacet, wax, etc.) than in mineral oil (corresponding to vaseline). This allows of the conclusion that the tendency of a phenylmercuric ointment to give off the phenylmercuric salt to the tissue juices is greater when the ointment consists

in vaseline than when it made up of lard or an other lipide substrate, provided that the ointments are of the same strength, that the phenylmercuric salt is *dissolved* in the unguentary substrate, and that ointments otherwise are equally able to mix and get in contact with the tissue juices. From the solubilities given in Table 1, however, it is not practicable to figure how the mentioned phenylmercuric salts will be distributed between the unguentary substrates and the tissue juices. In this respect one meets with some difficulties because the state of phenylmercuric salts differs according to the solvents (unguentary substrates, water, or tissue juices).

When phenylmercuric acetate is dissolved in mineral oil, olive oil or some unguentary substrate, it presumably is present as molecules of phenylmercuric acetate (but the possibility of association, for example, into double molecules cannot be excluded). In aqueous solution, on the other hand, the substance is found in three different forms: non-ionized phenylmercuric acetate (about 83%), ionized to phenylmercuric ions (about 2 %), and hydrolyzed to phenylmercuric hydroxide (about 15 %). According to Gjaldbæk & Helweg Mikkelsen, these figures apply to an aqueous solution of the strength 1:300 (0.01 molar).

As in aqueous solution the greater part of the phenylmercuric acetate (about 83 %) is present as phenylmercuric acetate molecules, it is possible from the figures in Table 1 approximately to calculate the distribution coefficient of this salt between water and, for instance, olive oil, finding it to be 40/250. But this distribution coefficient cannot be employed for calculation of the distribution of phenylmercuric acetate between olive oil and tissue juices, because tissue juices are buffers with p_H about 7. As phenylmercuric hydroxide is a very weak base ($K_B \approx \text{ca. } 10^{-11}$)²⁷, it may be calculated that phenylmercuric acetate in tissue juices with $p_H = \text{ca. } 7$ will be converted almost completely to phenylmercuric hydroxide; less than 1‰ will be present as phenylmercuric salt (non-ionized + ionized).

In consequence of the above-mentioned fact, that the tis-

sue juices will convert phenylmercuric salts almost completely to phenylmercuric hydroxide, the ointments which contain phenylmercuric salts in solution will be able to give off the phenylmercuric salt to the tissue juices to a far greater extent than suggested by the distribution coefficients that may be calculated from the figures in Table 1. For the same reason, presumably, it will be justified to discount the complication that the phenylmercuric salt might be precipitated as chloride — owing to the chlorides of the tissue juices. For, even though phenylmercuric chloride is almost insoluble in water, the possibility of chloride precipitation in the tissue juices will be slight, as phenylmercuric salts at $p_{H} = 7$ contain almost no phenylmercuric ions. It is reasonable to assume that phenylmercuric chloride is rather easily soluble in tissue juices.

So it seems hardly possible to prepare ointments containing phenylmercuric salts in such a way that the ointment has buffer and depot action as to the phenylmercuric salt. Even though the unguentary substrate is highly able to dissolve the phenylmercuric salt employed, the ointment will have a strong tendency to give off the phenylmercuric salt to the tissue juices; and on trituration of an almost water-insoluble phenylmercuric salt (for instance, the chloride) the ointment will also have a great tendency to give off the salt, because the salt is rather easily soluble in the tissue juices.

The idea then suggests itself to see whether it might be possible to prepare a phenylmercuric ointment with buffer action by employment of phenylmercuric hydroxide; but as this substance dissolves far more readily in water than do the phenylmercuric salts, the condition for its serviceability in ointment is the finding of an unguentary substrate that is highly capable of dissolving phenylmercuric hydroxide. The solubility of this hydroxide in water is about 1:80, and the unguentary substrate would therefore have to be rather able to dissolve about 10 % phenylmercuric hydroxide if it were to have any buffer action worth mention. In mineral oil and in olive oil, however, the solubility of phenylmercuric hydrox-

ide is less than 1‰, so that possibility of finding such a substrate appears to be very slight.

As it seems rather impracticable to prepare phenylmercuric ointments with buffer or depot action, it will hardly be possible to employ concentrated phenylmercuric ointments without causing irritation. In aqueous solution phenylmercuric salts are employed at a concentration of 1:1250 or as weaker solutions. In ointments, presumably, these salts may be employed in a somewhat, but not much, stronger concentration, because an ointment does not get into such an intimate contact with the tissues as does an aqueous solution; besides, it takes some time for the phenylmercuric compound to diffuse from the ointment to the tissues.

The experimental studies reported in the following were aimed to illustrate the ability of the phenylmercuric compound in different phenylmercuric ointments to diffuse from the ointment out into an agar plate. The experiments were made with the soluble phenylmercuric acetate and the almost insoluble phenylmercuric chloride incorporated in various unguentary substrates.

Technique.

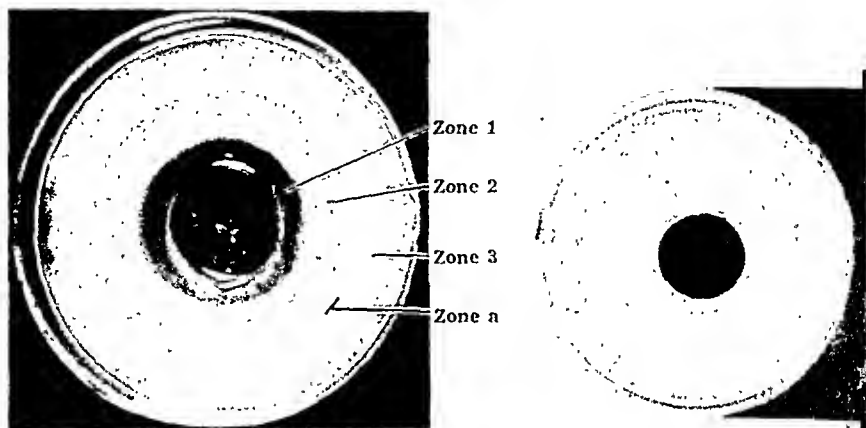
To elucidate the question about the diffusibility of phenylmercuric salts from different phenylmercuric ointments the following technique was employed.

Sterile ground glass rings, measuring 7 mm. in height and 2 cm. in internal diameter, are placed in the centre of sterile Petri dishes. Into each Petri dish, outside the glass ring, is poured 20 c. c. of melted 2% peptone agar (about 50°) thoroughly mixed with 5 drops of a 24-hour culture of *Microc. aureus*. This bacterium is chosen for test object because it is the most resistant of the more common non-spore-forming bacteria. When the agar is stiff, the glass ring is removed by means of sterile forceps, and about 3 g. of ointment is delivered from a tube into the empty circular hole in the plate left after the glass ring. If the ointment is hard (for instance, containing lanoline) it is first softened by standing in incubator for some length of time. If necessary, the ointment is brought into close contact with the agar wall of the hole by means of a sterile

glass spatula. Then the Petri dish is incubated for 36 hours at 37°, and photographed with a black paper for background.

A typical example of the results that may be obtained with this technique is seen in Fig. 1, which shows the result obtained with 1‰ phenylmercuric acetate in gum tragacanth and, for comparison, a control plate without ointment.

The dark circular area in the centre of the plate is the oint-



Gum tragacanth with 1‰
phenylmercuric acetate.

Control (no ointment in
the centre).

Fig. 1.

ment. The circular Zone 1 is clear, appearing like sterile agar medium, as there is no visible growth in this zone. Microscopic examination revealed the presence of a few solitary bacteria.

Zone 1, which is 8 mm. wide, is surrounded by Zone 2 (12 mm. wide) that shows macroscopic growth. Under the microscope, however, the growth in Zone 2 is found to be more scanty than the growth in the control dish. In the external Zone 3, the zone of normal growth, the density of the growth is the same as that in the control. At the transition between Zones 2 and 3 the microscopic examination reveals a very narrow band, Zone a, with particularly vigorous growth, even more abundant growth than in the normal zone and in the control (negative chemotaxis). For estimation of the diffusibility of the phenylmercuric compounds it will be more proper, no doubt, to base the estimate on the width of Zone 1, as this zone is permeated with so much phenylmercuric salt that the bacterial growth is inhibited.

Experiments.

Tests have been made with the following ointments, prepared by the Control Laboratory of the Danish Pharmaceutical Association:

- A. Phenylmercuric acetate 0.5 ‰ dissolved in adeps lanae, adeps lanae cum aqua, adeps lotus, dermatol, glycoderm, opa cream without perfume, ungv. molle, and vaseline.
- B. Phenylmercuric chloride 1 % triturated in the same unguentary substrates as employed under A.
- C. Phenylmercuric acetate ointments made of ungv. molle in the proportions 1.25 ‰, 2.5 ‰, 5 ‰, and 10 ‰.
- D. Phenylmercuric chloride ointments made of ungv. molle in the proportions 0.5 ‰, 1 ‰, 1.25 ‰, 2.5 ‰, 5 ‰, 25 ‰, and 50 ‰.

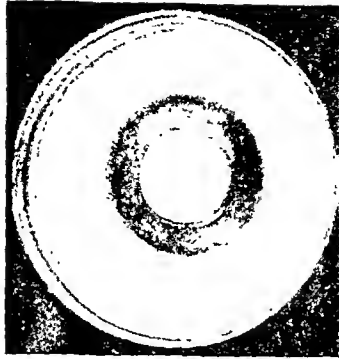
The results obtained in the experimental series A, B, C and D are given in Tables 2 and 3, recording the width of Zone 1 in mm.

Table 2.
Width of Zone 1 in mm.

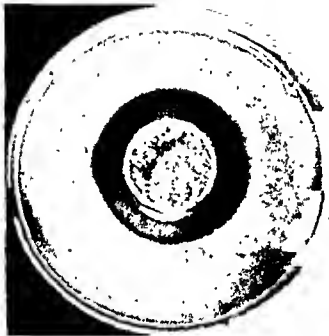
Unguentary substrate	A. Phenylmercuric acetate 0.5 ‰ sol.	B. Phenylmercuric chloride 1 ‰ triturat.
Adeps lanae	6.6	8.8
» » c. aqua	6.0	14.4
» lotus	7.6	10.4
Dermatol	7.6	9.6
Glycoderm	6.4	9.6
Opa cream without perfume	8.2	11.0
Ungv. molle	8.0	9.8
Vaseline	6.8	9.2

Table 3.
Phenylmercuric Ointment with Ungv. molle as Substrate.

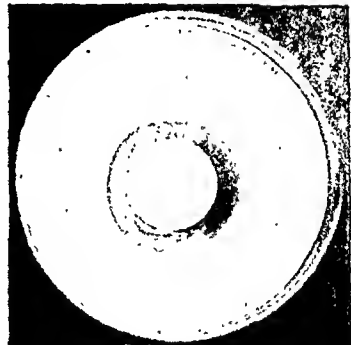
C. Phenylmercuric acetate		D. Phenylmercuric chloride	
Strength	Width of Zone 1 in mm.	Strength	Width of Zone 1 in mm.
1.25 ‰	6.6	0.5 ‰	5.8
2.5 ‰	(6.8)	1.0 ‰	6.8
5 ‰	6.8	1.25 ‰	6.8
10 ‰	8.4	2.5 ‰	7.0
		5 ‰	7.0
		25 ‰	7.0
		50 ‰	7.2



Opa cream without perfume,
with 0.5 ‰ phenylmercuric
acetate.



Ungv. molle with 0.5 ‰
phenylmercuric acetate.

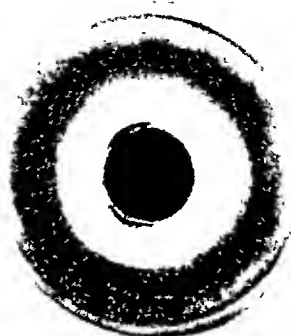


Glycoderm with 0.5 ‰ phe-
nylmercuric acetate.

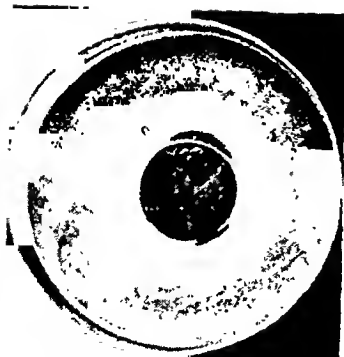
Fig. 2.

Table 2 shows that in Series A the width of Zone 1 has varied between 6.0 and 8.2 mm. Opa cream presents the widest zone; then ungv. molle. The narrowest zone is found for adeps lanae cum aqua. Strangely, in the ungv. molle ointment Zone 1 is wider than the corresponding zones in the components of ungv. molle — namely, vaseline and adeps lanae cum aqua. Still, there is no great difference in the width of the zones in the different experiments.

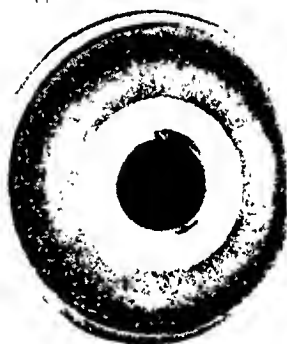
In Series B, adeps lanae cum aqua presents the widest Zone 1;



Opa cream without perfume, with 1 % phenylmercuric chloride.



Ungv. molle with 1 % phenylmercuric chloride.



Glycoderm with 1 % phenylmercuric chloride.

Fig. 3.

for ungv. molle width of Zone 1 is intermediate between the corresponding zones in adeps lanae cum aqua and vaseline. Opa cream shows a wide Zone 1 in this series too.

On comparison of the results in Series A and B it is found that a 1 % trituration of the chloride shows a wider Zone 1 than a 1 ‰ solution of the acetate.

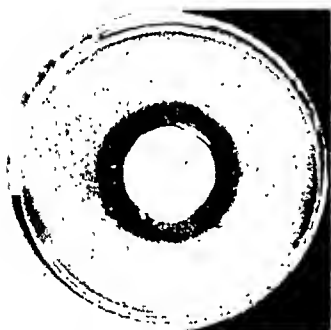
For illustration of the above experiments, some photos are shown in half size (Figs. 2 and 3).



Ungv. molle with 1.25 ‰
phenylmercuric acetate.



Ungv. molle with 10 ‰
phenylmercuric acetate.



Ungv. molle with 50 ‰
phenylmercuric chloride.



Ungv. molle with 0.5 ‰
phenylmercuric chloride.

Fig. 4.

The results of the tests in Series C appear to show that an increase in the concentration of phenylmercuric acetate does not essentially give a wider Zone 1; and the same applies to the chloride.

On comparison of Series C and D it appears as if a trituration of the chloride proves just as effective as a solution of the acetate in the same proportion.

A comparison of Series A and B with Series C and D would hardly be allowable, as Series A and B were not carried out at the same time as Series C and D and hence not with the same batch of agar. Some photos in half size illustrate the results (Fig. 4).

Conclusion.

The results show that phenylmercuric acetate ointments as well as phenylmercuric chloride ointments are bactericidal. The character of the unguentary substrate appears not to play any great rôle. Still, the diffusibility of the phenylmercuric salts here employed appears to be somewhat greater from opa cream and ungv. molle than from the other ointments. Trituration of the chloride seems to be just as effective as a solution of the acetate in the same proportion. On the other hand, the amount of acetate or chloride in the ointment appears to be of minor importance to the diffusibility of the salt from the ointment.

Summary.

A report is given of some experimental studies aimed to elucidate the question about the diffusibility of phenylmercuric salts incorporated in various unguentary substrates.

Description is given of the method employed for these tests.

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ON STERILIZATION OF 1 ‰ SOZOIODOLIC ACID SOLUTION.

By *Elsa Jensen.*

(Received for publication December 18th 1940).

The following experiments were carried out in order to ascertain whether a 1 ‰ solution of soziodolic acid is bactericidal, and thus look into what method of sterilization it has to undergo.

Respectively 10 c. c. of 1 ‰ soziodolic acid solution and 10 c. c. of sterile distilled water (for control) were placed in rubber-stoppered Erlenmeyer flasks, to which were added, by means of a Pasteur pipette, 5 drops of a 24-hour culture of *Micrococcus aureus*. The flasks were placed in incubator at 22° (room temperature). Subcultures were made with platinum loop, in broth, at once and after various intervals. After incubation at 37° for up to three weeks the results were read. The findings are given in Table 1.

Table 1.

Growth of Microc. aureus in Subcultures made from after Addition of 5 Drops of Broth Culture.

10 c.c. of	Subcultures made						
	at once	after 3 hrs.	6 hrs.	20 hrs.	24 hrs.	48 hrs.	3 days
1 ‰ soziodolic acid solution	+	+	+	0	0	0	0
Sterile distilled water	+	+	+	+	+	+	+

The experiment was repeated with addition of a suspension of soil spores from an agar-surface culture. The heat resistance of these spores at $p_H=8$, which according to Walbum¹⁾ is the hydrogenion concentration at which soil spores are most resistant, exceeded boiling for 10 hours. The results are given in Table 2.

Table 2.
Growth of Soil Spores in Subcultures made after Addition of 5 Drops of Suspension of Agar Surface Culture.

10 c.c. of	Subcultures made		
	at once	after 3 days	after 3 weeks
1 ‰ soziodolic acid sol.	+	+	+
Sterile distilled water	+	+	+

From the findings above it is evident that the micrococci in the soziodolic acid solution die out in less than one day, whereas they keep alive in the sterile distilled water, and that the soil spores are alive after two weeks' stay in the soziodolic acid solution and in the sterile distilled water. These findings are in keeping with some experiments previously reported by the writer.²⁾

Further, an experiment was made in which the soziodolic acid solution with addition of 5 drops of soil spore suspension was subjected to the heat treatment required in the Pharmacopoeia Danica 1933 with this variation, that subcultures were made in broth after various intervals and incubated at 37° for 3 weeks. Sterile distilled water was used for the controls in this experiment too, inoculated with 5 drops of the same soil spore suspension. The results are given in Tables 3 and 4.

Tables 3 and 4 show that 1 ‰ soziodolic acid solution possesses a relatively bactericidal power for the highly

¹⁾ Dansk Tidsskrift for Farmaci, 7: 37, 1933.

²⁾ Dansk Tidsskrift for Farmaci, 13: 1, 1939.

resistant soil spores here employed, as these spores keep alive in the sterile distilled water after heating, whereas they are killed in the chemical solution.

Table 3.

Growth of Soil Spores in Subcultures (5 Drops of Spore Suspension added to the Solution).

Heating to 100°.				
Cultures in Broth after	15 min.	30 min.	45 min.	1 hour
10 c.c. of 1 ‰ soziodolic acid solution	+	0	0	0
10 c.c. of sterile distilled water	+	+	+	+

Table 4.

Growth of Soil Spores in Subcultures (5 Drops of Spore Suspension added to the Solution).

Heating to 80°.				
Cultures in Broth after	30 min.	1 hour	1½ hrs.	2 hrs.
10 c.c. of 1 ‰ soziodolic acid solution	+	+	0	0
10 c.c. of sterile distilled water	+	+	+	+

Kromann Jensen³⁾ states that the *Dak* Laboratory (Control Laboratory of the Danish Pharmaceutical Association) has advised aseptic preparation of 1 ‰ soziodolic acid solution for injection. With a view studies previously reported by me⁴⁾, from which it was evident that only a few germs could be demonstrated on chemicals intended for the preparation of injection remedies, it will be safe, no doubt, in a great majority of instances to reckon with sterility of such remedies with aseptic precautions. But, as demonstrated in

³⁾ Archiv for Pharmaci og Chemi, 47: 229, 1940.

⁴⁾ Archiv for Pharmaci og Chemi, 43: 357, 1936; Zentralbl. f. Bakt. 138: 375, 1937.

Tables 3 and 4, it requires heating to obtain absolute sterility when the contaminating material consists in heat-resistant soil spores — namely, heating at 100° for at least 30 min. or at 80° for $1\frac{1}{2}$ hours.

From the experiments above, which confirm some previous experiments of mine⁴⁾, it appears, when considered from a bacteriological point of view, as if the pharmacopoeia is even very strict when it requires that a remedy prepared under aseptic precautions together with heating to 100° for one hour or to 80° for two hours is to be designated as sterile only after bacteriological control tests.

As the studies reported by Kromann Jensen⁵⁾ show that heating of a 1‰ soziodolic acid solution to 80° for 2 hours causes liberation of iodine only to slight extent as corresponds to decomposition of 0.7% of the soziodolic acid, this form of treatment has to be considered the more suitable for sterilization of 1‰ soziodolic acid solutions.

According to Dr. Geill⁵⁾ the therapeutic effect of the iodine liberated under this treatment must be said to be of minor value.

The studies here reported were carried out at the suggestion of the Control Laboratory of the Danish Pharmaceutical Association.

⁵⁾ Private communication.

THE A ANTIGEN CONTENT OF RABBIT SERUM AND ITS IMPORTANCE FOR THE FORMATION OF A ANTIBODY.

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In a previous paper it was mentioned that rabbits owing to their capability to develop antibody by immunization with human blood corpuscles of type A can be divided into two more or less equal, well characterized groups. The animals of the one group respond to such immunization by developing, besides species antibody, a small quantity of or, possibly, no A agglutinin, and no sheep hemolysin, whereas the animals of the other group produce a great quantity of A agglutinin and also a great quantity of sheep hemolysin.

In order to explain this difference in the capability to develop antibody *Dölter* in 1925 suggested that A antigen must be contained in serum or organs of the first named group of animals (which, in his opinion, are quite unable to respond to immunization with A red corpuscles by developing specific antibody). That this supposition was essentially correct was subsequently proved experimentally by several authors. Thus, *Witebsky* with the aid of experiments of fixation of the complement with alcoholic extract of lung and kidney from one of two rabbits detected A antigen, without paralleling this finding however with the capability of the animals to produce antibody. *Hara* and *Treibmann* com-

pared the quantity of A antigen contained in the serum of rabbits with the quantity of preformed A agglutinin, and thus found that those sera which contained no preformed A agglutinin did contain A antigen which, on the other hand, failed to be detected in sera containing preformed A antibody. These authors determined the A antigen content of serum by examining whether the serum was capable of binding the sheep hemolysin in a conveniently diluted anti-A serum, and thus of preventing hemolysis by the addition of complement and sheep red corpuscles.

However, on applying this fairly complicated method so many factors assert themselves that it is often difficult to estimate the results, which have actually been rather incalculable for other researchers.

Nor is the content of preformed A agglutinin in serum a reliable expression of the capability of the animals to develop antibody, since virtually all the animals contain some A antibody at any rate. Thus, I on examining my experimental animals found that the titer for the preformed A agglutinin varied between 0 and 128. Those animals which had strong preformed antibody were *as a rule* capable of developing strong A antibody also after immunization, while those with weak preformed A agglutinin very seldom had this property. However, a sharp line of distinction could not be drawn, for even animals with the low titer of 2 were capable of developing antibody, whereas animals with a titer of 16 were unable to do so (Table 1).

Table 1.

Titer	< 4	4	8	16	32	64	128	Altogether
Much anti-A	3	7	8	3	5	2	1	29
Little or no anti-A	17	4	3	1				25
Altogether	20	11	11	4	5	2	1	54

The Table shows the distribution of titer for the preformed A agglutinin among those rabbits which, after immunization, produce strong A antibody (upper row), and among those which are not able to do so (middle row).

Mai and *Torben Andersen* examined the A antigen content of extracts of lung and kidney and that of serum, respectively, and compared the result with the capability of the animals of developing antibody, and they found in the main that those animals in which A antigen was detected were unable to develop A antibody, while the others were capable of doing so.

These different experiments thus indicate on the whole that there is a relation between the capability of the animals to develop antibody and their A antigen content. Since my experiments of immunization described in the preceding paper illustrate so distinctly the varying capability of the animals of producing antibody, I endeavoured to discover a corresponding characteristic difference in the antigen content.

Whereas the mentioned authors in their studies mostly used the inhibition of the hemolysis of sheep red corpuscles for measuring the A antigen content of a serum, I preferred, on account of the many drawbacks encumbering that method, a modification of the precipitation and absorption method devised by *Oluf Thomsen*. With the aid of this method the protein substances of the serum are precipitated after addition of acetic acid and heating. The precipitate is used for absorption of an immune serum, and comparison between the titers of this serum recorded prior and subsequent to absorption affords a measure and identification of the antibody which the precipitate has been able to bind. This method is simpler and affords more perspicuous results than does the inhibition of the hemolysis of sheep blood. Besides, the latter method only affords information with regard to A antigen of the same kind as that contained in sheep red corpuscles. The precipitation method on the contrary affords the possibility of obtaining information regarding other parts of the A antigen too, as immune serum before and after absorption with the precipitate can be tested by titration both in experiments of agglutination, of hemolysis and of complement fixation.

Technic: 0.4 cc. of that serum whose A antigen content shall be examined is diluted with 1.6 cc. of a 0.9 % salt solution, and 2 drops of 1 % acetic acid are added. The tube is then placed on a constant water-bath of 80° C. for 15 minutes, and shaken frequently. Thereby a fine precipitate appears. After centrifugation the limpid fluid is removed, and the precipitate is washed once with 0.9 % saline. To the precipitate is then added 0.4 cc. of the conveniently diluted anti-A serum to be absorbed, this serum previous having been absorbed with O corpuscles. The precipitate being mixed carefully with serum, the mixture is left to stand at room temperature for absorption, as a rule for 1 hour. Finally, the absorbed serum is compared with the unabsorbed serum with the aid of titration. The technic used for the titrations was described in the previous paper.

That this method affords precipitation of antigen together with the albuminous substances in such a manner that, by absorption, a specific fixation of antigen and antibody ensues, is shown by the experiment recorded in Table 2.

The rabbit R40 is immunized with A blood corpuscles and has thereby developed strong A agglutinin and sheep hemolysin. Serum from this rabbit is absorbed with O blood corpuscles and, afterwards, with precipitates from human O serum, human A serum, and serum from two rabbits (R59 and R61); serum R40 is then tested by titration of hemolysin opposite sheep red corpuscles (S61) and of agglutinin opposite A blood corpuscles.

The first section of the Table shows that the precipitate of A serum and of R61 serum caused a very considerable reduction of the titer, whereas the other two sera absorbed a minor quantity of antibody. The absorption brought about by human O serum must at any rate be unspecific, for, as serum R40 had previously been absorbed with O blood corpuscles, no O antibody can have been left to be removed. Nor does the absorption of R59 exceed the limit of unspecific absorption, whereas that absorption which is caused by human A serum and R61 serum must be considered to be caused by A antigen. The experiment moreover shows that this absorption is not complete, for in that case the A serum ought to have removed all the sheep hemolysin. By stronger, eventually repeated, absorption it will be possible to remove more antibody, but then the unspecific reaction will also become stronger, and that is not very desirable.

The agglutination experiment shows quite the same proportion as the hemolysis experiment.

The last section of the Table represents a corresponding experiment performed with serum from a rabbit (R34) immunized with

Table 2.

				1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192
R40 abs. with O		~	S61:	100	100	100	100	100	100	100	60	20
" " " " + prec. A	~	~	" :	100	100	80	40	20	0			0
" " " " + " O	~	~	" :	100	100	100	100	100	100	40	20	0
" " " " + " R59	~	~	" :	100	100	100	100	100	80	40	20	0
" " " " + " R61	~	~	" :	100	100	100	60	20	0			0

				1/32	1/64	1/128	1/256	1/512
R40 abs. with O	~	A ₁ :	++ ++	+	+	(+)	0	
" " " " + prec. A	~	" :	++ ++	0				
" " " " + " O	~	" :	++ ++	+	+	0		
" " " " + " R59	~	" :	++ ++	+	(+)	0		
" " " " + " R61	~	" :	++ ++	0				

				1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384	1/32768
R34 abs. with O		~	S61:	100	100	100	100	100	100	100	100	80	40
" " " " + prec. A	~	~	" :	100	100	100	100	100	100	60	40	20	0
" " " " + " O	~	~	" :	100	100	100	100	100	100	100	40	20	0
" " " " + " R59	~	~	" :	100	100	100	100	100	100	80	60	20	0
" " " " + " R61	~	~	" :	100	100	100	100	100	100	80	40	20	0

Examples of experiments illustrating the specificity of the precipitation method.

sheep red corpuscles. This rabbit was not capable of developing sheep hemolysin after immunization with A blood corpuscles, whence that sheep hemolysin which formed after immunization with sheep blood corpuscles contained no A antibody. (Therefore, no agglutination experiments were made with A blood corpuscles).

This serum was absorbed with the same precipitates as serum R40, but in this experiment quite uniform, weak absorption was observed. It must therefore be warrantable to presume that the 4 precipitates have given rise to an unspecific fixation of the sheep

antibody. This unspecific fixation probably is due to simple adsorption to the comminuted precipitate.

Such slight, unspecific fixation is also observed when an immune serum is absorbed with serum precipitate from the same animal, as seen in Table 3.

Table 3.

			1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384	1/32768	
R44 abs. with O	~	S61:	100	100	100	100	80	60	40	20	0
" " " " + prec. R44	~	" :	100	100	100	100	80	40	20	0	
" " " " + " R46	~	" :	100	100	100	100	60	40	20	0	
" " " " + " R43	~	" :	80	40	20	0					

In this experiment serum from R44 is absorbed with precipitate both from the animal's own serum and from sera of 2 other rabbits (R46 and R43). Whereas the precipitate from R43 affords very strong absorption, that of the other two animals is so faint that it must be considered unspecific.

The accuracy of the method is evidenced by the experiment recorded in Table 4.

Table 4.

			1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384	1/32768	
R42 abs. with O	~	S61:	100	100	100	100	100	100	100	60	20	0
R42 abs. with O + prec. R52(1)	~	" :	100	100	100	100	80	80	60	40	20	0
" " " " + " " (2)	~	" :	100	100	100	100	100	80	60	40	20	0
" " " " + " " (3)	~	" :	100	100	100	100	100	80	40	20	20	0
" " " " + " " (4)	~	" :	100	100	100	100	100	80	60	40	20	0
" " " " + " " (5)	~	" :	100	100	100	100	100	60	40	20	20	0
R42 abs. with O + prec. R51(1)	~	" :	100	100	100	60	40	20	0			
" " " " + " " (2)	~	" :	100	100	100	40	20	0				
" " " " + " " (3)	~	" :	100	100	60	40	40	0				
" " " " + " " (4)	~	" :	100	100	100	60	40	0				
" " " " + " " (5)	~	" :	100	100	100	60	40	20	0			

Experiment showing the accuracy of the precipitation method.

Serum from R42, which is immunized with A blood corpuscles, is absorbed with precipitates of 5 portions of serum from R50 and of 5 portions of serum from R51. After absorption, the unabsorbed serum and the 10 portions of absorbed serum were titrated opposite sheep red corpuscles. From the Table it is evident that the precipitates from R50 afforded a faint, probably unspecific absorption, whereas those from R51 afforded strong absorption. However, the 5 specimens from each serum virtually yielded identical results.

Thus, these experiments show that this method is so accurate, and that the difference between the faint, unspecific reaction and the strong, specific reaction is so great, that it must be applicable for demonstrating A antigen in serum.

Now the first task will be to examine in what rabbits A antigen can be demonstrated in serum in this manner.

In Table 5 a, b, c, d are recorded 4 experiments which comprise all the rabbits I disposed of at the time when the

Table 5 a.

			1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384	1/32768	1/65536
R44 abs. with O	~ S61:		100	100	100	100	100	100	80	60	40	20
R44 abs. with O + prec.	R3 ~ » :		100	100	100	100	100	100	60	40	20	0
» » » » + »	R8 ~ » :		—	—	80	80	80	60	40	20	0 ^{*)}	
» » » » + »	R11 ~ » :		100	100	100	100	100	60	40	20	0	
» » » » + »	R12 ~ » :		100	100	100	100	100	80	60	20	20	0
» » » » + »	R20 ~ » :		100	100	100	100	100	80	60	20	0	
» » » » + »	R21 ~ » :		100	100	100	100	100	80	40	20	0	
» » » » + »	R23 ~ » :		100	100	100	100	80	60	40	20	20	0
» » » » + »	R24 ~ » :		100	100	100	100	80	60	40	20	20	0
» » » » + »	R27 ~ » :		100	100	100	100	100	80	40	20	0	
» » » » + »	R28 ~ » :		100	100	100	100	100	60	40	20	0	
» » » » + »	R5 ~ ~ » :		100	100	80	40	20	0				
» » » » + »	R14 ~ » :		100	100	100	40	20	0				
» » » » + »	R16 ~ » :		100	100	100	80	40	20	0			
» » » » + »	R18 ~ » :		100	100	100	40	20	20	0			

^{*)} This experiment partly failed, because the tube was broken. its content thus being spilt.

Table 5 b.

			1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384	1/32768	1/65536
R44 abs. with O	~ S61:		100	100	100	100	100	100	100	80	60	40
R44 abs. with O + prec.	R32	~	100	100	100	100	100	100	100	80	60	40
» » » » + »	R33	~	100	100	100	100	100	100	100	80	60	20
» » » » + »	R36	~	100	100	100	100	100	100	100	80	60	40
» » » » + »	R40	~	100	100	100	100	100	100	100	60	40	20
» » » » + »	R42	~	100	100	100	100	100	80	80	60	40	20
» » » » + »	R31	~	100	100	100	100	60	20	0			
» » » » + »	R34	~	100	100	80	60	40	0				
» » » » + »	R35	~	100	100	100	100	80	60	40	0		
» » » » + »	R37	~	100	100	100	100	80	60	40	0		
» » » » + »	R38	~	100	100	80	80	60	60	40	20	0	
» » » » + »	R39	~	100	100	100	100	60	60	40	20	0	
» » » » + »	R41	~	100	100	100	80	100	60	20	0		

Table 5 c.

			1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192
R42 abs. with O	~ S61:		100	100	100	100	100	100	100	100	80	60
R42 abs. with O + prec.	R44	~	100	100	100	100	100	100	100	100	40	20
» » » » + »	R46	~	100	100	100	100	100	100	100	80	60	20
» » » » + »	R48	~	100	100	100	100	100	100	100	40	20	0
» » » » + »	R49	~	100	100	100	100	100	100	100	80	40	0
» » » » + »	R50	~	100	100	100	100	100	100	100	100	60	40
» » » » + »	R43	~	100	100	100	80	40	20	0			
» » » » + »	R45	~	100	100	100	100	100	60	20	0		
» » » » + »	R47	~	100	100	100	100	80	60	40	20	0	
» » » » + »	R51	~	100	100	100	100	80	40	20	0		
» » » » + »	R52	~	100	100	100	80	40	20	0			

Table 5 d.

Table 6.													
			1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	1/16384	1/32768	1/65536	
R44 abs. with O			~ S61:	100	100	100	100	100	100	60	40	20	0
R44 abs. with O + prec.			R53 ~ » :	100	100	100	100	100	100	60	40	0	
»	»	» » + »	R57 ~ » :	100	100	100	100	100	80	60	0		
»	»	» » + »	R58 ~ » :	100	100	100	100	100	80	60	20	20	0
»	»	» » + »	R59 ~ » :	100	100	100	100	100	80	40	20	0	
»	»	» » + »	R54 ~ » :	100	80	80	60	40	20	0			
»	»	» » + »	R55 ~ » :	100	100	80	40	20	0				
»	»	» » + »	R56 ~ » :	100	100	80	40	20	0				
»	»	» » + »	R60 ~ » :	100	100	100	60	40	0				
»	»	» » + »	R61 ~ » :	80	60	40	20	0					
»	»	» » + »	R62 ~ » :	80	60	40	20	0					

The Table shows absorption experiments with serum precipitates from all the animals tested in this manner. The precipitates from the different animals have either a very slight or a distinct capability of binding the sheep hemolysin in an anti-A serum.

experiments were carried out. 36 of the animals were tested opposite serum from R44, and 10 of them opposite serum from R42. Both these animals were immunized with A blood corpuscles.

It is seen that, for each experiment, the Table is divided into 2 sections, the uppermost section comprising those rabbits whose serum precipitate afforded but slight absorption, and the lowermost section comprising those affording stronger absorption.

Now it is evident that the absorption recorded for those precipitates which are quoted in the uppermost sections of the Tables do not in any one case exceed what, according to the experiments, may be considered unspecific absorption. It is true that this absorption is not quite constant, partly because there is some difference between the different experiments — Table 5 b for example showing averagely

less unspecific fixation than Table 5 d — and partly because there is also some difference between the fixation of the several precipitates within the same experiment. Nor can it be excluded that this variation is partly due to a specific factor concurring in some cases, but it must be considered impossible with regard to these absorptions to distinguish more accurately between specific and unspecific absorption.

On the other hand, looking at the absorption of those sera which are recorded in the second section of the Tables, it proves to be so strong in all the cases that it cannot be attributed to unspecific fixation alone, but it must be assumed that the fixation is due chiefly to these sera containing A antigen. However, the A antigen demonstrated in that way corresponds only to a part of the A antigen contained in human blood corpuscles, namely, that part which is contained in sheep red corpuscles also.

The difference between the two groups of rabbits is so great that it must be warrantable to conclude that distinction must be made between a group of rabbits with little or no A antigen in the serum and a group of rabbits with distinctly demonstrable A antigen.

Now on comparing the distribution obtained in this way with that derived from immunization of the rabbits, which is described in a previous paper (Tables 1 and 2, p. 340), it becomes evident that there is perfect agreement, for *all those animals in whose serum no A antigen was detected were capable, after immunization, of developing strong sheep hemolysin, whereas none of those whose serum contained A antigen was capable of producing such antibody.*

The same partition into two groups according to the absorptive power of the precipitates was observed in numerous experiments and on using different immune sera, whence it can be established that there does exist a difference in the A antigen content of the animals corresponding to their capability of developing antibody.

On using different anti-A sera from different rabbits for absorption in these experiments it became evident that,

whereas some of these sera always were absorbed very strongly and specifically, others were absorbed very feebly, the absorption thus exceeding but very little the limit for unspecific absorption. A similar difference was observed, when sera were used, which were taken from the same animal at different moments during the immunization.

However, this difference in the capability of anti-sera of being absorbed was quite constant, regardless of what precipitate was used, and it was also observed when the total A antigen, such as it is contained in A blood corpuscles, was used for the absorption. In Table 6 is recorded an experiment which shows that, by absorption with A blood corpuscles, very little of the antibody contained in serum from R24

Table 6.

				1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240	1/20480
R24 abs. with O	~	S61:	100	100	100	100	100	100	100	100	60	40	20
» » » » + 1/500 vol. A ₁	~	» :	100	100	100	100	100	100	100	80	60	40	20
» » » » + 1/200 » »	~	» :	100	100	100	100	100	100	100	80	20	0	
» » » » + 1/100 » »	~	» :	100	100	100	100	100	60	20	0			
» » » » + 1/60 » »	~	» :	100	100	100	60	40	20	0				
R42 abs. with O	~	S61:	100	100	100	100	100	60	60	40	20	0	
» » » » + 1/500 vol. A ₁	~	» :	100	100	100	80	60	40	0				
» » » » + 1/200 » »	~	» :	100	80	60	40	20	0					
» » » » + 1/100 » »	~	» :	40	20	20	0							
» » » » + 1/60 » »	~	» :	0										
R44 abs. with O	~	S61:	100	100	100	100	100	100	80	60	40	20	0
» » » » + 1/500 vol. A ₁	~	» :	100	100	100	100	80	60	60	40	20	0	
» » » » + 1/200 » »	~	» :	100	100	100	80	60	40	20	0			
» » » » + 1/100 » »	~	» :	80	60	40	20	20	0					
» » » » + 1/60 » »	~	» :	0										

Example showing that different quantities of A blood corpuscles are required for the removal of sheep hemolysin from different anti-A sera.

is removed, whereas the antibody contained in serum from R42 and R44 is absorbed far more easily.

These experiments indicate that a difference in the avidity of the antibodies is responsible for the absorption of antibodies in the different anti-A sera not being the same.

In a previous paper it was described that the A antigen contained in human red corpuscles consists of several qualitatively different fractions, one fraction thus being a Forssman antigen proper which is found as a species-specific property in all sheep and also in organs of guinea pigs. Moreover, some sheep contain other fractions of the A antigen in different quantities.

That A antigen, which is demonstrated in rabbit serum, corresponds, as was mentioned p. 425, to such A antigen as is contained in sheep red corpuscles, since absorption of sheep hemolysin was used for demonstrating it. Therefore; it is of interest to examine whether (1) this antigen corresponds both to that which, as a Forssman antigen proper, is contained in the blood corpuscles of all sheep, and to such A antigen as is contained in the blood corpuscles of some sheep only, and (2) whether rabbit serum may be found to contain A antigen which is not contained in sheep red corpuscles; finally, (3) whether a qualitative difference can be demonstrated in the A antigen content of the different sera.

Whether rabbit sera can contain *A antigen which is also found as a species-specific property in the blood corpuscles of all sheep* was examined in the following manner: An anti-A serum prior and subsequent to absorption with a number of serum precipitates was titrated with sheep red corpuscles, which solely or chiefly contained this A antigen (S49). An example of such an experiment is recorded in Table 7.

Whether rabbit sera can contain *such A antigens as are found — in greater or smaller quantities — in the blood corpuscles of some sheep only* was examined as follows: An anti-A serum prior to absorption with the precipitates was absorbed with sheep blood corpuscles solely or chiefly

Table 7.

				1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096		
R42 abs. with O				~ S49:	100	100	100	100	100	80	60	20	0
»	»	»	» + prec. R44	~ » :	100	100	100	80	40	20	0		
»	»	»	» + » R46	~ » :	100	100	100	80	60	20	0		
»	»	»	» + » R48	~ » :	100	100	100	100	80	40	20	0	
»	»	»	» + » R49	~ » :	100	100	100	100	100	100	60	20	0
»	»	»	» + » R50	~ » :	100	100	100	100	60	20	20	0	
»	»	»	» + » R43	~ » :	100	100	40	20	0				
»	»	»	» + » R45	~ » :	100	100	60	20	0				
»	»	»	» + » R47	~ » :	100	100	100	60	40	20	0		
»	»	»	» + » R51	~ » :	100	80	80	60	40	20	0		
»	»	»	» + » R52	~ » :	100	80	40	20	0				

Experiment showing that some rabbit sera contain such A antigen fractions as are also contained in the blood corpuscles of *all* sheep.

Table 8.

				1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192	
R42 abs. with O + S49 + prec. R44	~	S61:		100	100	100	100	100	60	40	20	0	
» » » » + » + » R46	~	» :		100	100	100	100	100	80	80	60	20	0
» » » » + » + » R48	~	» :		100	100	100	100	80	40	20	0		
» » » » + » + » R49	~	» :		100	100	100	100	80	40	20	0		
» » » » + » + » R50	~	» :		100	100	100	100	100	60	20	0		
» » » » + » + » R43	~	» :		100	80	60	20	0					
» » » » + » + » R45	~	» :		100	100	60	40	20	0				
» » » » + » + » R47	~	» :		100	100	80	40	20	0				
» » » » + » + » R51	~	» :		100	60	40	20	0					
» » » » + » + » R52	~	» :		100	100	100	40	20	0				

Experiment showing that some rabbit sera contain those A antigen fractions which are contained in the blood corpuscles of some sheep only.

containing that A antigen which is common for all sheep (S49). Thereby the corresponding hemolysins are removed, whereas those hemolysins, which correspond to the A antigens found in some sheep only, are left in the serum. Afterwards it is examined whether the precipitates are capable of removing these hemolysins. Such an experiment is recorded in Table 8. For the experiments recorded in Table 7 and Table 8 the same anti-A serum (R42) was used. Looking, at first, at the lowermost group in the Tables, it is seen that both experiments present distinct absorption, which is most pronounced in Table 8 however. *The sera of these 5 rabbits thus contain such A antigen as is found in all sheep as well as such A antigen as is found in some sheep only.* There is some difference in the absorptive power of the different precipitates; this is also evidenced by other experiments (Table 5), but it is scarcely possible to decide whether that is due to qualitative or quantitative differences. None of these animals was capable of producing sheep hemolysin after immunization.

In the uppermost section of the Tables a rather slight absorption is noted, but in Table 7 the absorption recorded for R44, R46 and — though in a somewhat lesser degree — R50 is so strong that it can hardly be considered to be solely unspecific. This might indicate that these sera actually contained some of the »common« A antigen which is also found in the blood corpuscles of all sheep.

After immunization with A blood corpuscles all the animals in the uppermost section developed strong sheep hemolysin, both corresponding to the »common« A antigen and to the »special« A antigen which is contained in the blood corpuscles of some sheep only. However, on examining the proportion — in the immune sera of these animals — between these »common« and the »special« antibody fractions, it becomes evident that there are variations here, too, which correspond, at any rate in some degree, to the differences in the absorptive power of the serum precipitates.

Table 9.

		1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192
R44 abs. with O	~ S49:	100	100	100	100	60	40	20	0
» » » » + S49	~ S61:	100	100	100	100	80	40	20	0
R46 abs. with O	~ S49:	100	100	80	60	40	20	0	
» » » » + S49	~ S61:	100	100	100	100	80	60	40	20 0
R48 abs. with O	~ S49:	100	100	80	60	20	20	0	
» » » » + S49	~ S61:	100	100	80	60	40	20	0	
R49 abs. with O	~ S49:	100	100	80	60	40	0		
» » » » + S49	~ S61:	100	100	80	60	40	20	0	
R50 abs. with O	~ S49:	60	40	20	20	0			
» » » » + S49	~ S61:	100	100	100	60	20	0		

Experiments showing a qualitatively varying composition of the sheep hemolysin in anti-A sera.

Thus in Table 9 are recorded titrations of antibody opposite the »common« A antigens (titrated with S49 blood corpuscles) and of antibody opposite the »special« A antigens (titrated with S61 blood corpuscles after absorption of the serum with S49 blood corpuscles). The serum was taken when the animals had been immunized 5 times with A blood corpuscles.

The Table shows that, both with regard to R46 and R50, the titer for the »common« antibody was much lower than for the »special« antibody, whereas these two titers were equally high with regard to the other animals — and also R44. Examinations performed at other moments during the immunization revealed similar conditions. This indicates that the capability of R46 and R50 of developing antibody opposite the »common« A antigen is relatively inferior, and that corresponds to the serum precipitates from these animals affording a fairly strong absorption of the »common« anti-

body; such an agreement is not found with regard to R44 however.

The experiment shows, however, that it can scarcely be assumed that the sera of those animals which, after immunization with A blood corpuscles, are capable of developing sheep hemolysin, do not contain any A antigen at all. It must sooner be assumed that they contain minor quantities which are not, however, able to prevent the formation of antibody but which are able to influence its composition.

Finally it was examined whether rabbit serum contains such A antigen as is *not* contained in sheep red corpuscles. This was done by absorption of an anti-A serum (R42) with sheep red corpuscles containing as much A antigen as possible (S61).

Even though one cannot be sure that blood corpuscles from this sheep have been able to remove all the antibodies opposite that A antigen which may be contained in sheep red corpuscles, it must be assumed that the remaining antibody chiefly corresponds to that A antigen which is *not* contained in sheep red corpuscles. Afterwards this serum was absorbed with different serum precipitates and, finally, titrated in experiments of agglutination with A blood corpuscles.

Such an experiment is recorded in Table 10. Precipitates from R43, R45, R51 and R52, but not from R47, afforded a stronger absorption than the precipitates from the other 5 animals, which indicates that the sera of these 4 rabbits contain this antigen fraction. However, the difference is not great, and no difference was found in other experiments.

After immunization with A blood corpuscles virtually all the animals formed agglutinin opposite this antigen fraction though in very different quantities. Those animals, which produced no sheep hemolysin, formed but very little A agglutinin, whereas the other animals developed strong agglutinin opposite this fraction.

Table 10.

						1/16	1/32	1/64	1/128
R42 abs. with O + S61 +	~	A ₁ :	+	+	(+)	0			
» » » » + » + prec. R44	~	»:	+	(+)	0				
» » » » + » + » R46	~	»:	+	+	0				
» » » » + » + » R48	~	»:	+	(+)	0				
» » » » + » + » R49	~	»:	+	+	0				
» » » » + » + » R50	~	»:	+	+	0				
» » » » + » + » R43	~	»:	+	0					
» » » » + » + » R45	~	»:	+	0					
» » » » + » + » R47	~	»:	+	+	0				
» » » » + » + » R51	~	»:	+	0					
» » » » + » + » R52	~	»:	+	0					

This experiment (Table 10) tends to suggest that there is a corresponding variation in the content of this A antigen fraction in sera, but reliable information in this respect has not been derived from these experiments.

Conclusion. These experiments thus show that there is a difference in the A antigen content of rabbit serum, which corresponds to their different capability of developing antibody after immunization.

All those rabbits which, after immunization with A blood corpuscles, are capable of producing but little A agglutinin and no sheep hemolysin, present A antigen in serum; this A antigen consists chiefly — if not solely — of those fractions which are contained in sheep red corpuscles.

On the other hand, the sera of those rabbits which, after immunization with A blood corpuscles, produce strong A agglutinin and sheep hemolysin, contain on the whole a very small quantity of or, possibly, no A antigen at all. Certain experiments indicate that these animals may contain a very small quantity of that A antigen which is also found in the

blood corpuscles of all sheep, and that such small quantities of antigen are responsible for even strong immune sera not having the same qualitative composition.

Summary.

In a previous paper it was shown that rabbits according to their capability of developing antibody after immunization with A blood corpuscles can be divided into two more or less equal groups, the animals of the one group only being able to produce very little A agglutinin and no sheep hemolysin, whereas those of the other group produce strong A agglutinin and sheep hemolysin.

In the present work it was examined whether the difference in the capability of developing antibody is due to differences in the A antigen content of the sera of the animals.

In these experiments the different sera after dilution and addition of acetic acid were heated to 80° C. Thereby strong precipitation was produced, and the precipitate was used for the absorption of an anti-A serum.

Examinations of sera from 46 rabbits performed in that manner unmistakably revealed A antigen in 22 of them, whereas the sera of 24 other animals practically contained no A antigen.

Comparison of the capability of these animals to develop antibody revealed that none of the first named 22 animals after immunization with A blood corpuscles was capable of producing sheep hemolysin, and that they were able to produce very little or no A agglutinin at all. On the other hand, all the other 24 animals whose serum on the whole contained no A antigen, produced strong A antibody.

However, certain experiments indicate that the sera of these animals may contain a small quantity of that A antigen which, as a real Forssman property, is contained in the blood corpuscles of all sheep.

It must therefore be assumed that the cause of the different capability of producing antibody detected in these animals is a constitutional difference in the A antigen content of the sera.

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DANSK PATOLOGFORENINGS 1. MØDE*) 27/3-1941
I KØBENHAVN.

*The first Meeting of the Danish Pathological Society,
March 27, 1941.*

Première séance des pathologistes danois, le 27 mars 1941.

Erste dänische Pathologentagung am 27 März 1941.

H. C. Bendixen: *Über Vakzination gegen Rinderbrucellose mit lebenden Brucellabakterien von geschwächter Virulenz.*

In 26 chronisch infizierten Beständen mit 3363 Milchkühen wur-

*) Dansk Patologforening, der er en Underafdeling af Nordisk Patologforening, har hidtil kun holdt Møder i Forbindelse med denne. Foreningen er nu sluttet sammen med den path.-anatomiske Sektion af Dansk biologisk Selskab og holder fremtidig selvstændige Møder. Referater af disse vil blive offentliggjort i Acta pathologica et microbiologica scandinavica.

The Danish Pathological Society, which is a subsection of the Nordic Pathological Society, has heretofore held meetings only in connection with the latter. The Society is now united with the pathologico-anatomical section of the Danish Biological Society, and will henceforth hold independent meetings. Reports from such meetings will be published in Acta pathologica et microbiologica scandinavica.

Les pathologistes danois, qui représentent une subdivision de la Société de pathologistes nordiques, ont jusqu'ici siégé conjointement avec ces derniers. La Société, s'étant réunie avec la section pathologique-anatomique de la Société de Biologie danoise, tiendra dorénavant des séances indépendantes. Les comptes rendus de ces séances seront publiés dans Acta pathologica et microbiologica scandinavica.

Der dänische Pathologenverein, eine Unterabteilung des Nordischen Pathologenvereins, hat bislang nur in Verbindung mit diesem getagt. Der Verein, der nunmehr mit der path.-anatomischen Sektion der dänischen biologischen Gesellschaft zusammengeschlossen ist, wird künftighin selbständige Tagungen abhalten. Berichte von solchen werden in Acta pathologica et microbiologica scandinavica erscheinen.

den Blutproben auf Agglutiningehalt untersucht. Folgende Serumverdünnungen: 1:10, 1:20, 1:40, 1:100 und 1:200 wurden angewandt. Tiere, die totale Agglutination in einer Verdünnung 1:10 und darüber zeigten, wurden als Reagenten (R) bezeichnet. Vakzination ist in einigen der Bestände (1267 Kühe) systematisch in der Weise durchgeführt worden, dass die Tiere als Färsen ungefähr 10–8 Wochen vor Decken mit lebender Kultur geimpft wurden. 2096 Kühe sind nicht vakziniert worden.

	Anzahl Kühe	p.Ct. R	p.Ct. der Reagenten mit positivem Titer				
			1 : 200	1 : 100	1 : 40	1 : 20	1 : 10
Nicht geimpfte Tiere	2096	44,4	34,6	11,1	17,9	17,9	18,5
Geimpfte Tiere	1267	26,8	15,9	8,8	18,2	22,1	35,0

Aus der Tabelle geht hervor, dass die Reaktionsverhältnisse bei vakzinierten Kühen sich viel günstiger gestalten als bei den nicht vakzinierten. Besonders ist die Anzahl von Reagenten mit hohen Titern viel kleiner bei den geimpften Tieren.

In einem der untersuchten Bestände, der 130 Milchkühe enthielt und durch eigene Aufzucht ergänzt wurde, sind die Färsen ca. 15 Jahre hindurch systematisch geimpft worden; in den letzten Jahren wurden die Färsen in einem Alter von 10–12 Monaten geimpft. Dieser Bestand wurde jährlich ein- bis zweimal untersucht. Im Jahre 1937 wurden 23,3 % Reagenten, im Dezember 1940 nur 6,5 % gefunden.

Durch Impfung an Meerschweinchen liess sich feststellen, dass die Milch der Kühe, die nur einmal gekalbt hatten, keine Brucellabakterien als Folge der Vakzination enthielt. Nach Ausmerzen von 8 Reagenten mit hohem Agglutinationstiter im Laufe von 1939–40 war die Mischmilch des Bestandes frei von Brucellabakterien, jedenfalls konnten die Bakterien nicht durch Meerschweinchenimpfung nachgewiesen werden. Der Bestand konnte zu der Zeit mit geringen Kosten unschwer ganz von Reagenten befreit werden. Indessen hat man bis jetzt noch nicht mit der Impfung nachgelassen, weil die Gefahr besteht, dass der Bestand von Nachbarbeständen neu infiziert werden könnte.

Der benutzte Bakterienstamm hat in besonderen Meerschweinchenversuchen einen ähnlichen Grad von herabgesetzter Virulenz gezeigt wie der amerikanische Stamm N 19 (Bendixen u. Jørgensen, Jahrbuch Kgl. Veterinär- und landwirtschaftliche Hochschule 1940, pag 80). Bei diesen Untersuchungen wurde konstatiert, dass der Verlauf der Infektion bei den vakzinierten Meerschweinchen

bedeutend milder als bei nicht vakzierten verlief, indem die Tendenz zur Generalisation und damit hohe Agglutinationstiter stark herabgesetzt war. Dasselbe scheint in ähnlicher Weise beim Rind der Fall zu sein, indem ein kleineres Prozent der vakzierten Tiere permanente Infektionen bekommen und damit wird die Anzahl der Bakterienausscheider oder die Anzahl von Tieren mit dauernd hohem Agglutinationstiter wesentlich herabgesetzt.

Systematische Vakzination in grösseren Beständen trägt dazu bei, dass die ökonomischen Verluste, welche die Brucellose verursacht, bedeutend vermindert werden. Es hat sich aber ausserdem gezeigt, dass die Anzahl von Kühen mit dauernd hohem Agglutinationstiter sowie die Anzahl von Bacillenausscheidern stark vermindert wird, welches für das Endziel: die Tilgung der Seuche, von wesentlicher Bedeutung ist.

Diskussion: J. Ørskov, P. Kragh.

Knud Sand und Harald Okkels: *Über die Struktur des Hodens bei gesetzlich kastrierten Personen.*

Das dänische Gesetz über Zulassung zur Sterilisation und Kastration hat durch die Bestimmung, dass operativ entfernte Hoden an das gerichtsmmedizinische Universitäts-Institut einzusenden sind, mit sich geführt, dass während der verlaufenen 12 Jahre eine grosse Anzahl Hoden von — abgesehen von der Sexualabnormität — körperlich meist gesunden Männern nach und nach zur histologischen Bearbeitung gesammelt wurde.

Unser Kontrollmaterial stammt von Sektionen durch Unfall oder plötzlich gestorbener Männer, bzw. Selbstmörder, bei denen die Autopsie sonst keine ausgesprochenen krankhaften Zustände zeigte.

Bis zum Februar 1941 sind 112 Paar Hoden von gesetzlich Kastrierten und 121 Kontrollhoden mikroskopiert und beschrieben worden. Jedem Hoden wurden 2—4 Gewebescheiben winkelvecht zur Längsachse des Organs entnommen und aus jeder Gewebescheibe wurden viele Präparate angefertigt, die routinemässig mit Heidenhains Eisenhämatoxylin gefärbt und danach verschiedenen Spezialmethoden, insbesondere der Osmium- und Silberinprägnation unterzogen wurden.

Wir sind jetzt in unserer Forschung so weit gekommen, dass wir die durch Mikroskopie erzielten Ergebnisse der Hoden von 200 Individuen, 100 aus jeder Kategorie, Kastraten, bzw. Kontrollen, miteinander vergleichen können. In unserer Zusammenstellung ist der morphologische Status der beiden in biologischer Beziehung spezifischen Gewebekomponenten des Hodens ausgedrückt; 1) Der Zustand des *intratubulären* (spermatogenen) Epithels; und 2) Die Menge des *spezifischen, intertubulären* Gewebes: die Leydig-Zellen.

Die Beurteilung der histologischen Befunde der von unseren 100 Kastraten und 100 Kontrollen herrührenden Hoden ergab, dass in der Kastratengruppe ein sicheres Übergewicht innerhalb der Gruppen vorhanden ist, die wohl als pathologisch zu bezeichnen sind. Dieses Übergewicht ist besonders ausgesprochen in den Gruppen, die die Mengenverhältnisse der Leydig-Zellen ausdrücken.

Bei Berechnung gibt das Kontrollmaterial einen Mittelwert, der einem individuellen Gesamthodengewicht von 40 g. entspricht. Das durchschnittliche Gewicht der Kastratengruppe liegt ein wenig höher. Betrachten wir das Material derartig eingeteilt, so zeigt sich, dass bei den kleinen Hoden ein Übergewicht innerhalb der Kastratengruppe mit Bezug auf die Reduktion der Spermatogenese vorhanden ist; anscheinend ist dies auch der Fall, was die Leydig-Zellen anbelangt. Diese Ausschläge sind aber erheblich deutlicher, wenn wir die Gruppe der grossen Hoden betrachten. Schon dies dürfte wohl als ein Ausdruck dafür betrachtet werden, dass die Abweichungen auf *absoluten Verschiebungen* beruhen. Wir glauben daher schon zum gegenwärtigen Zeitpunkt nachgewiesen zu haben, dass in den Geschlechtsdrüsen gesetzlich Kastrierter strukturelle Änderungen vorliegen, und zwar teils gekennzeichnet durch eine verhältnismässig stärkere Verödung des spermatogenen Epithels — ganz besonders aber durch das Übergewicht der Vermehrung der Leydig-Zellen in quantitativer Beziehung.

Inga Scheibel: *The Importance of Selection and Inbreeding on the Capability of Guinea-Pigs to Produce Diphtheria Antitoxin.**)

Among an incidental population of guinea-pigs previously employed in immunizing experiments with diphtheria anatoxin, a selection was made of males and females which had not produced measurable quantities of diphtheria antitoxin, and others which had produced ample quantities. The two series of animals were mutually mated, with the idea of obtaining a division into a good and a bad antitoxin-producing strain by means of selection and inbreeding. Their progeny (F1) were immunized with 10 Fl. units of diphtheria anatoxin, and four weeks later a blood sample was taken by heart puncture; the antitoxin content in the samples was measured by intracutaneous titration on rabbits. For the F2 group a selection was made from the F1 group of the good and the bad strains, taking those animals which had the highest and the lowest antitoxin content respectively. The same procedure was continued through five or six generations — as far as possible by mating brother and sister.

*) The paper will be published later in extenso in Acta path. et microbiol. scand.

It was found that with the good strain the selection resulted in almost 100 per cent. of antibody-producers even in F1, and that this percentage continued practically unchanged through the generations, though it was not possible to demonstrate any increase in the antitoxin titer, whereas the bad strain revealed a gradual decrease of antitoxin producers from 54 per cent. in F1 to 11 per cent. in F5 and a corresponding fall in the antitoxin titer.

These experiments justify the assumption that the capacity of producing diphtheria antitoxin and also the absence of that capacity as far as guinea-pigs are concerned are chiefly hereditary; they also indicate a predominance of the antitoxin-producing disposition.

Discussion:

Johns. Ipsen, T. Kemp.

Fridtjof Bang: *Twenty Cases of Reticulosarcoma and a special form of this Tumor («with Clear Cells»)*. With a Histological Examination of 109 Malignant Tumors of Tonsils and Rhinopharynx.

The material comes from the Radium Station, Copenhagen, from the period of 1930—40, and comprises 109 cases of malignant tumors of the tonsils and rhinopharynx in 78 men and 31 women. Of these tumors 52 originated from the epithelium of the mucous membrane (none of these tumors showed any cornification). Two of the tumors were transitional-cell carcinomata, 25 were lympho-epitheliomata, and 3 lympho-sarcomata; various other tumors were represented by 6 cases. Reticulosarcoma was represented by 20 cases (+ one that was somewhat doubtful).

The classification of the reticulosarcomata is based on the differentiation between syncytial and dictyocytic forms. Of the *dictyocytic* forms some were clear-cut, while in other cases there was an admixture of lymphocytes or plasma cells. No standpoint is taken as to the question whether this admixture is due to differentiation or to a sort of symbiosis. Attention is called to the fact that these forms resemble the changes encountered in mycosis fungoides, and it is reported that one of the patients with a tumor of the rhinopharynx was suffering from mycosis fungoides. The question whether the latter lesion be of neoplastic nature is mentioned briefly. In two of the cases the tumors were polymorpho-cellular; one of these patients had also tumors of the small intestine, corresponding to the observations reported by Ahlström.

The *syncytial* reticulosarcomata appeared in two forms: 1) entirely syncytial, and 2) a particular form designated as »reticulosarcoma with clear cells«. The latter form shows syncytial streaks, along the margin of which there appear some large, round, clear cells with a central dark-staining nucleus, which often shows mitosis. The clear cells form notches in the syncytia. The nature of these cells cannot be established precisely. Also this form of tumor ap-

pears in a »pure« form as well as with an admixture of other cells, especially dictyocytes, lymphocytes and Albertini bodies. In the latter cases the tumor is extremely rich in cells and nuclei, so that its appearance is quite in keeping with a designation sometimes applied to reticulosarcomata, namely: a »jumble of cells«. Among these cells it is possible, especially in very thin sections, to demonstrate the aforementioned peculiar round, clear cells.

With Foot's staining this form of tumor shows a large amount of reticular fibres, and invasive growth (e.g. into the adjacent musculature).

The course of illness in patients with this form of tumor indicates — at any rate, for the present — that the tumor is very sensitive to radiation.

Two additional cases are mentioned, in which this tumor appeared in the form of ulcerations of the palate.

Arne Gammelgaard: *Über Serumfälle. Ein Todesfall nach prophylaktischer Tetanusantitoxininjektion.*

Der Vortrag wird später in Acta path. et microbiol. scand. veröffentlicht werden.

Diskussion:

J. Ørskov, Th. Madsen, V. Friedenreich, L. Heerup.

Carl Jakobsen: *Ein Fall von Enterokystom.*

Als Ursache von Ileus bei einer 24jährigen Frau wurde ein walnussgrosses, intramurales Enterokystom gefunden, das etwa 30 cm von der Ileocoecalclappe entfernt auf der Mesenterialseite der Ileumwand entwickelt war.

Mikroskopisch erwies sich der Tumor zwischen den beiden Muskelschichten der Darmwand gelegen. Seine Wand bestand aus typischer Dünndarmschleimhaut, einer schwächeren und einer dickeren Schicht glatter Muskelfasern. Meistens waren eine oder mehrere Schichten degeneriert und von einer Art Granulationsgewebe ersetzt. In der Nähe des Tumors fanden sich in der Darmsubmucosa mehrere kleine Cysten, die aus Dünndarmschleimhaut und einer Schicht glatter Muskelfasern aufgebaut waren.

Die Enterokystome sind zum ersten Male im Jahre 1881 von Roth beschrieben worden; es liegen jetzt etwa 150 kasuistische Mitteilungen vor; aus diesen geht hervor, dass die Enterokystome in der Darmwand von Pylorus bis Rektum entwickelt sein können, dass sie auch ausserhalb des Darms, zwischen den Blättern des Mesenteriums, im Mediastinum, in der vorderen Bauchwand in der Nähe des Nabels und auch multipel auftreten können. Die Grösse wird von Nuss- bis Männerkopfgrösse angegeben. Das mikroskopische Bild ist sehr verschieden; gemeinsam ist die grössere oder geringere Ähnlichkeit mit der Darmwandstruktur.

Betreffs des Ursprungs sind viele Theorien aufgestellt worden; jetzt sind die meisten Verfasser geneigt, die Enterokystome teils als Überreste des Ductus omphalo-mesentericus, teils als Abkömmlinge von fötalen Proliferationen des Darmepithels zu betrachten. Die letztere Genese hat wahrscheinlich der beobachtete Fall.

Diskussion:

P. Møller.

H. C. Mømberg-Jørgensen: *Zur Infektion mit Salmonella typhi murium bei Gänsen.*

Während Salmonellainfektionen bei Enten mehrfach nachgewiesen worden sind, sind Berichte über das Vorkommen von Salmonellainfektion bei Gänsen in Dänemark äusserst spärlich, speziell scheint die Infektion bei ausgewachsenen Gänsen früher nicht nachgewiesen zu sein.

Bei der Untersuchung von 228 Blutproben von Gänsen auf einem Gut in Jütland zeigten 10 positive H-Agglutination gegenüber *Salmonella typhi murium* und die serologische Diagnose wurde durch Sektion und bakterielle Untersuchung von 2 der Reagenten bestätigt. Bei der Sektion wurden verschiedene grössere und kleinere, etwas eckige Follikel mit teilweise inspissiertem Inhalt gefunden, die durch kürzere oder längere Bindegewebsstränge mit dem Ovarium verbunden waren; andere Follikel waren völlig vom Ovarium getrennt oder lagen frei in der Bauchhöhle oder waren durch Bindegewebe mit Darmschlingen verwachsen. Der Sektionsbefund war also ganz analog dem, den man bei Salmonellainfektionen bei Enten und Hühnern erhebt. Trotz eingehender bakteriologischer Untersuchung gelang es nur in den pathologisch veränderten Follikeln, aber sonst in keinem anderen Organ *Salmonella typhi murium* nachzuweisen.

In bezug auf die serologischen Verhältnisse der isolierten Stämme sei angeführt, dass sowohl VI als auch V O-Antigen vorgefunden wurde; kulturell waren die Stämme u. a. dadurch charakterisiert, dass sie langsam rhamnose- und inositvergärend, d-Tartrat-negativ, Na-Citrat-negativ sowie Stern-positiv waren.

Neben den Gänsen hatte das Gut einen Bestand von 101 Hühnern, von denen 18 positive Seroreaktion gegen *Salmonella typhi murium* gaben. Auf welche Weise die Infektion auf dem Gut entstanden war, wurde nicht ermittelt; in den letzten 6 Jahren waren dort weder Bruteier noch Geflügel eingekauft worden. Eine grössere Sterblichkeit wurde unter den Gänsehen nicht beobachtet, obgleich in der letzten Brutperiode mehr als 3000 Gänsehen in 28 Brutmaschinen ausgebrütet wurden. So viel man weiss, sind auf dem Gut keine Menschen mit der Krankheit angesteckt worden.

Diskussion:

Martin Kristensen, Harhoff.

DANSK PATOLOGFORENINGS 2. MØDE 7/6-1941
I KØBENHAVN.

*The second Meeting of the Danish Pathological Society,
June 7, 1941.*

Seconde séance des pathologistes danois, le 7 juin 1941.

Zweite dänische Pathologentagung am 7. Juni 1941.

J. P. Sjolte: *Primäre maligne Geschwülste in den Lungen bei Tieren.*

Es werden besonders die primären Carcinome besprochen, da Sarkome so selten sind, dass hier nicht näher auf diese eingegangen wird. Im Schrifttum sind im ganzen 85 Fälle von primärem Carcinom in den Lungen besprochen, und von diesen sind 49 Fälle näher beschrieben. Betrachtet man die Zeit von 1880—1940 in zehnjährigen Zeitabschnitten, so ist eine merkbare Steigerung der Häufigkeit der beschriebenen Fälle festzustellen. Die Häufigkeit ist am grössten beim Pferd und Hund, was wohl mit dem Milieu zusammenhängt, in welchem diese Tiere leben, da das Rind sie ja zahlenmässig übertrifft. Doch muss angenommen werden, dass das Alter eine Rolle spielt — eine geringere Anzahl Rinder erreichen ein ebenso hohes Alter wie Pferd und Hund. Aus der Tatsache, dass im Schrifttum fast ausnahmslos Fälle bei älteren oder alten Tieren beschrieben sind, geht hervor, dass das Alter eine Rolle spielt. Über den Einfluss des Geschlechts gibt das Schrifttum keine Auskunft, was mit Hinblick auf das Interesse, welches sich an die Feststellung der Häufigkeit des Vorkommens von Tumoren bei Kastraten knüpft, zu bedauern ist (Hormonstörung?). Anatomisch findet man entweder einen primären Tumor mit oder ohne Metastasen oder primäre multiple Geschwülste. Bei Pferd und Hund herrscht ein einzelner primärer Tumor vor, bei Rind und Schaf multiple primäre Geschwülste. Histologisch wechselt der Grad der Ausdifferenzierung des Geschwulstepithels sehr.

Das eigene Material des Verfassers umfasst 20 Fälle: 9 beim Hund, 3 beim Pferd, 7 beim Rind und 1 bei einem Jaguar. Anscheinend nimmt die Häufigkeit in den letzten Jahren zu. Alle Tiere waren älter oder alt. Das Geschlecht schien dagegen keine Rolle zu spielen. Makroskopisch wurde das im Schrifttum beschriebene Bild gefunden. Mikroskopisch fand der Verfasser auch Carcinosarkome. In einem Falle hatte ein Carcinosarkom reine Carcinommetastasen in der Milz und reine Sarkommetastasen in den Nieren hervorgerufen.

(Die Arbeit wird später in extenso veröffentlicht).

Lorentz Heerup: »*Bacteriological Autopsies in Human Typhoid-Paratyphoid Infections.*

In animal experiments Ørskov and cooperators have shown the probable »mechanism of infection« by aseptic autopsies with bacteriological examination of samples from blood and various organs from mice killed at varying intervals after oral infection with bacilli of the salmonella-group.

By similar »bacteriological autopsies« in cases of various mortal infections in man the author has tried to elucidate the course and actual state of infection.

By »bacteriological autopsy« is meant the absolutely aseptic taking of samples from blood, fluids and organs for aerobic and anaerobic bacteriological cultures with the organs *in situ* before the usual autopsy is performed.

In this way 8 cases of typhoid-paratyphoid infection in man were examined, 3 cases of typhoid and 5 cases of paratyphoid infection.

In only 2 of these cases, both of them paratyphoid infections, a diffuse bacteriemic sepsis was found, with culture of b.paratyphi B from all the samples taken; in the one case the patient was a puerpera, in the other case (diagnostized clinically as »cryptogenic sepsis«) a canalicular, suppurative pyelonephritis was found due to an obturating ureteral calculus.

In the other 6 cases the infection was localized to certain organs of the reticulo-endothelial system (r. e. s), especially the liver, spleen and lymphatic glands.

The »bacteriological autopsies« seem to indicate that the »mechanism of infection« in man runs a similar course as in the animal experiments:

The infection takes place from the intestinal tract where the microorganisms infect the lymphatic tissues and pass on to the mesenteric glands.

From here they get access to the blood *via* the thoracic duct

causing an »initial bacteriemia« or a periodical or more constant »cyclic bacteriemia«, during which they often can be cultivated from the blood or the roseola *in vivo*. The microorganisms are quickly captured and retained by the r.e.s., — most constantly the spleen, the various lymphatic glands (probably also the tonsils) and the liver, — in a slighter degree also in the kidneys and the lungs, and so disappear from the circulating blood.

In these organs the fight between the microorganisms and the infected organism is fought. Almost generally the r.e.s.-barrière of the liver is broken and the bile and the gallbladder gets infected. The infected bile reinfects the intestinal tract which meanwhile rather early has been cleansed from the microorganisms of the primary infection. Clinically it is often seen that the intestinal content on the 5th—7th day of the infection does not contain any of the specific microorganisms but does so on later examinations. In the same way the kidneys are assumed to behave like the liver.

As a rule the infection is overcome by the r.e.s., eventually assisted by humoral factors, but in other cases the resistance of the organism is lowered by various factors or a specific infectious focus is developed and then a secondary bacteriemia arises, — a real bacteriemic diffuse sepsis.

This happened as mentioned in 2 of the reported cases, while the infection in the other cases was halted in the r. e. s. and death occurred as the result of hemorrhages or various secondary non-specific infections, especially pneumonias due to pneumo-, strepto- or staphylococci.

The most constant and massive growth of the specific microorganisms was always found in the mesenteric glands and the spleen (not so constantly in the gallbladder), and it is therefore recommended to remove samples from these organs for bacteriological examinations under absolutely aseptic conditions before the exvisceration is performed in cases suspect for typhoid-paratyphoid infections.

Discussion:

Ørskov: It is a pleasure to see that laboratory work acquires practical importance. The mentioned investigations might be systematized both in human and veterinary medicine. Dr. H. forgot to mention the importance of the infection of the upper lymphatic glands. Thinks, as Heerup, that coli bacilli by »incarceration« give rise to general infection, for example in case of renal calculi.

Svend Petri, Jens Bing, Ejnar Nielsen & Aage Kjerbye Nielsen: *Deficiency of the antipernicious anemia*

principle in the livers of gastrectomized hogs (climicotherapeutical study).

Altogether 8 pernicious-anemic patients have been treated with liver extracts from one total-gastrectomized and three electively fundus-resected swine. This treatment lasted from 5—13 days; as a rule the daily dose was 5 cc., less frequently 2 cc. For control the patients were treated with ordinary hepsol (MCO), this treatment being given immediately after the discontinuance of the experimental. Also the antipernicious-anemic effect of extracts from the liver of two normal swine was tested on 2 pernicious-anemic patients.

Total gastrectomy performed on the pig 11 months before had brought about a complete absence of antipernicious-anemic principle in the liver. This confirms the findings reported by Bence and by Goodman, Geiger & Claiborn.

In 3 pigs — with an observation period of 116, 258 and 379 days, respectively — elective resection of the fundus had also caused a marked reduction in, or complete loss of, the antipernicious-anemic principle in the liver. With these experiments there is demonstrated for the first time to exist a relation of dependency between the function of the fundus region and the amount of specific principle in the liver.

The view is advanced that the fundus of the stomach is associated with a specific function of importance to the utilization of the *extrinsic factor* in the organism.

Discussion:

Sjövall thanks Petri and co-workers for the great work, which, according to the lecture, has progressed considerably. Queries whether further progress with regard to experimental induction of pernicious anemia may be anticipated, since the animals, after deprivation of the antipernicious anemia principle, merely incur a deficiency disease (experimental endogenous pellagra) but no real pernicious anemia, and whether the animals do not lack that property of reaction which human beings own.

Petri thanks Sjövall for his remarks and interest as well as for previous assistance in examining the changes in the central nervous system of gastrectomized pellagrose experimental animals. Agrees with Sj. that, as yet, it has not been possible to induce typical pernicious anemia, even though the latest, very comprehensive ventriculo-intestinal resections on pups have given rise to a morbid condition strongly reminding of pernicious anemia. Is of opinion that the biological apparatus of the experimental animals is such as to render induction of experimental pernicious anemia possible under adequate experimental conditions. Reminds of the

fact that pernicious anemia in Man is observed exceptionally only after ventricle resections. The possibility must be taken into consideration that the prevailing conception of the causal relations of pernicious anemia is fallacious.

J. Ørskov: *Neue Untersuchungen über die Genese der Blutkörperchen und Blutplättchen.*

In einer früheren Arbeit*) über denselben Gegenstand glaube ich den Wahrscheinlichkeitsbeweis geführt zu haben, dass die Blutplättchen nicht von den Megakaryocyten gebildet werden, sondern mehr oder weniger entleerte Normoblastkerne oder Bruchstücke derselben darstellen. Besonders eine Reihe von Vergiftungsbildern, die durch kombinierte Anwendung von Phenylhydrazin und Bleichlorid hervorgerufen werden, liessen sich nach meiner Ansicht auf keine andere Weise deuten. Ich habe später diese Vergiftungsversuche mit genau dem gleichen Ergebnis wiederholt.

In allen untersuchten Säugetieren, Mäusen, Kaninchen, Meer-schweinchen, Pferden, Kühen und Katzen, finden wir verschieden aussehende Normoblastkerne, entweder in Form einer intakten Kugel oder eines mehr oder weniger geteilten, fragmentierten Körpers, der 2—4, ja zuweilen 6 Kernteile repräsentiert. Bei dieser Fragmentation nimmt die Kernmasse an Volumen oder Massigkeit nicht ab, und alle Angaben über intrazelluläre allmähliche Auflösung des Kernes sind unrichtig. Falls der Kern intrazellulär verschwindet, kann es nur durch eine plötzliche totale Auflösung ohne Hinterlassung der geringsten Spur morphologisch nachweisbarer Kernreste geschehen. Wenn man bedenkt, ein wie bastanter Körper der Normoblastkern ist, so ist diese letzte Erklärung ganz unwahrscheinlich. Neben diesen intrazellulären Kernen sehen wir oft im Marke selbst nach schonender Präparation eine grössere oder geringere Anzahl mehr oder weniger luxierter kugelförmiger oder fragmentierter Kerne. Ich nehme an, dass diese Kernluxation in dieser Form meistens ein abnormes Produkt ist, das indessen eine Tendenz zeigt, wie sie keine anderen Markzellen besitzen. Bei dieser Kernluxation entstehen keine Körper, die den Blutplättchen gleichen, und alle Versuche, die Blutplättchenbildung direkt zu verfolgen, waren vergebens. Bei der Katze ist es relativ leicht, sich zwischen den Zellkernen und den Blutplättchen einen Zusammenhang vorzustellen, da die Blutplättchen der Katze mindestens doppelt so voluminös wie die anderer Säugetiere sind. Aber erst bei den erwähnten Vergiftungsversuchen, wo wir eine abnorme Blutgenese

*) Die Genese der roten Blutkörperchen und Blutplättchen bei Säugetieren. *Folia Haematologica*, Bd. 59, S. 145, 1938.

verursachen, tritt der Zusammenhang zweifelsfrei zu Tage. Wir rufen hier eine starke Anämie hervor und gleichzeitig eine totale Veränderung der meisten neugebildeten Blutplättchen, indem diese grösser und massiver werden und das Blutbild die gleichmässigsten Übergänge von intrazellulär liegenden Kernen zu relativ entleerten Kernteilen zeigt, die an den roten Blutkörperchen festhängen, und weiter zu freien Blutplättchen von Kerngrösse bis herab zu kleinen Blutplättchen normaler Grösse. Gleichzeitig sieht man oft eine charakteristische Vakuolisierung, die sowohl Kerne wie Blutplättchen befällt.

Eine weitere morphologische Erscheinung im Knochenmark, die in dieser Beziehung auffällig ist, ist die Tatsache, dass sich im normalen Knochenmark immer erstaunlich wenig Blutplättchen vorfinden, ein Umstand, der im ersten Augenblick überrascht. Wenn man bei der Markpunktur in der Klinik oft viel Blutplättchen im Knochenmark findet, so liegt dies daran, dass man bei der Punktur eine Blutung hervorruft und dadurch eine abnorme Anhäufung von Plättchen erhält. Dies erklärt sich wohl daraus, dass sich die jüngsten Reticulocyten länger im Knochenmark aufhalten als die neugebildeten Plättchen. Bei gewissen Tieren, z. B. Mäusen und Kaninchen, lassen sich mit den angewendeten technischen Verfahren die Reticulocyten geringster Reife z. B. in Formolpräparat direkt an ihrer Form nachweisen. Sie sind unregelmässig gebogen, wie ein gedrehter Teigkloss, oder wie ein »Knoten«, wie Lisa Boström es nennt. Man fühlt sich versucht, in diesen Fällen von Markreticulocyten zu sprechen. Im übrigen scheint Grund für die Annahme vorzuliegen, dass die Zahl der Reticulocyten im peripheren Blut möglicherweise unabhängig ist von den vom Knochenmark ausgestossenen Blutkörperchen, da man sich vorstellen kann, dass die Aufenthaltszeit der Reticulocyten im Mark beträchtlich variiert. Mit geeigneter Technik ist es möglich, die Reticulocyten je nach der Menge von gefärbtem Netzwerk in die 3 Gruppen I, II und III einzuteilen. Gruppe I repräsentiert die roten Blutkörperchen, die nur äusserst spärliche Netzzeichnung enthalten, Gruppe III zeigt ein grobes Netz, das oft die ganze Zelle ausfüllt, und Gruppe II liegt in der Mitte zwischen den ersten beiden. Beim Auszählen der verschiedenen Typen findet man eine gleichmässige Verteilung im peripheren Blute, während Typus III das entsprechende Blutbild im Knochenmarke ganz beherrscht, wobei zugleich im Knochenmarke der prozentische Anteil im Marke immer weit grösser als im Blute ist. Dies lässt sich nur so deuten, dass die jüngsten vom Kerne befreiten roten Blutkörperchen eine gewisse, vielleicht sehr variable Aufenthaltszeit im Knochenmarke haben, ehe sie in das periphere Blut übergelien, und dies heisst wieder, dass sich die

Blutplättchen im Knochenmarke nicht so lange aufhalten wie die neumodellierten roten Blutkörperchen. Die Folge davon wäre natürlich, dass die Blutplättchen in einem zufälligen Markpräparat relativ spärlich vertreten wären im Vergleich mit den vorhandenen roten Blutkörperchen.

Technik: Fixierung und Aufschwemmung von Mark und Blut in 10 % Formolsalzwasser. p_H 7,6.

Retieuloeytdemonstration: Aufschwemmung in folgender Flüssigkeit: Homologes Serum 1 Teil, physiologisches Salzwasser 9, Glycerin 0,25 Teile. Färbung mit Brillantkresylblau. Etwas Farbpulver wird in physiologischem Salzwasser aufgelöst und ein passender Tropfen dem Suspensionstropfen auf grossem Objektglas zugesetzt. Der Farbzusatz muss je nach dem Präparat so abgestimmt werden, dass keine nennenswerten extrazellulären Ausfällungen stattfinden. Bedeckung mit grossem Deckglas. Kerndarstellung: Zusatz von 1 % Salzsäure.

Diskussion: Fähræus arbeitet in Upsala mit den nämlichen Problemen. Die Markretieuloeyten agglutinieren nicht mit den übrigen Blutkörperchen. Sie treten besonders nach Blutungen auf. Die Markreticulocyten sind die ersten Indikatoren der Blutregeneration. Sie sind am besten in frischem Blut zu sehen. »Das Netz« bewegt sich.

Ørskov dankt Fähræus. Die Markretieuloeyten werden im peripheren Blute nur bei jungen Tieren angetroffen.

Poul V. Mareussen: *Erzeugung von Thrombopenie und Megakaryocytose durch experimentelle Polycytämie, ein Beitrag zur Beleuchtung der Blutplättchenbildung.*

Der Zweck der vorliegenden Versuchsreihe war, zu untersuchen, inwieweit zwischen der Erythro- und der Thrombopoiese eine feste Beziehung besteht. Als Methode wurde experimentelle Polycytämie bei Kaninehen angewandt, die durch tägliche Transfusionen von gruppenverträglichem Blut hervorgerufen wurde. Bei den drei Versuchstieren trat in 2—3 Wochen, nach einer vorübergehenden Thromboeytose, eine ausgeprägte Thrombopenie ein, bei einem der Versuchstiere mit einer erhöhten Blutungstendenz aus Venenläsionen. Der Grad der Thrombopenie zeigte eine direkte Abhängigkeit von der Polycytämie, die erstere trat jedoch etwas später ein. Die Thrombocytenkurve verlief in groben Zügen parallel mit der Anzahl der erythrogeneren Zellen im Knochenmark, sodass die Thrombopenie gleichzeitig mit der maximalen Reduktion der Normoblastenzahl im Knochenmark eintrat. Während der Thrombopenie war die Zahl der Megakaryocyten im Knochenmark stark vermehrt, und ebenso die Zahl der myelogenen Zellen. Die Ver-

suche deuten auf eine nahe Beziehung zwischen der Erythro- und der Thrombopoiese hin und lassen sich ohne Schwierigkeit von den Gesichtspunkten aus erklären, die Ørskov geltend gemacht hat, wogegen sie nach den bisherigen Erfahrungen des Verfassers nicht mit Wright's Blutplättchentheorie in Übereinstimmung gebracht werden können.

Diskussion: Lindau betrachtet M.s. Methode mit den wiederholten Injektionen als elegant. Findet es auffallend, dass die Theorien so gut zutreffen. Die Ergebnisse bestätigen Ørskovs Theorie. Die Frage muss wiederaufgenommen werden. Die Megakaryocyten-theorie ist in den letzten Jahren allein herrschend gewesen. Man muss mit Ørskovs und Marcussens Theorie weiterarbeiten.

Gormsen: Untersucht man das Sternalpunktat von Patienten mit Thrombopenie, so findet man die Megakaryocytenzahl erhöht. Bei Normalen sind im Protoplasma von etwa 25 % der Megakaryocyten Blutplättchen-elemente enthalten, bei Thrombopeniepatienten weniger. Nach Splenektomie nimmt die Zahl der Wright'schen Figuren in den folgenden Tagen zu.

Petri beglückwünscht den »Konzern Ørskov-Marcussen« zu den Untersuchungsergebnissen, die besonders erfreulich seien für den der seit Jahren als einziger behauptet habe, Wrights Theorie sei falsch. Viele, insbesondere italienische Untersucher haben sich die Ähnlichkeit zwischen der Protoplasmastruktur der Megakaryocyten und der Blutplättchen und der Adhaesion der Blutplättchen an die Megakaryocyten (*in Ausstrichpräparaten*) für die Wright'sche Theorie zunutze gemacht. Unter Hinweis auf die mannigfache traditionelle Art und Weise, in der die numerische Nichtübereinstimmung im Laufe der Zeit wegerklärt worden ist, empfiehlt Petri, eine Differentialzählung der Megakaryocyten (*in Schnittpräparaten*) besonders im Hinblick auf die Zahl der Wright'schen Figuren auszuführen.

Ørskov empfiehlt Dr. Gormsen, das Sternalpunktat von chirurgischen Patienten, bei denen Knochen entfernt worden sind, zu untersuchen. Wenn es den Experimenten entspricht, werden wenig Blutplättchen vorhanden sein. Es ist nicht zu vergessen, dass die Megakaryocyten das labilste Element im Organismus sind. Es sind oft Kunstprodukte.

Marcussen dankt Lindau, Petri, Gormsen und lenkt die Aufmerksamkeit darauf, dass 3 Versuche nur einen vorläufigen Bericht gestatten. Die Megakaryocyten lassen sich in Schnittpräparaten schwer zählen. Das Material ist zu klein, um endgültige Rückschlüsse daraus abzuleiten.

F. Kauffmann: *Zur Serologie der Dysenterie Flexner-Gruppe*.

Es wurden serologische Untersuchungen an den Typen A, D und H von *Kruse*, sowie den englischen Typen VZ, WX, W, Z und Hiss-Russell Y ausgeführt, um zu einer praktisch brauchbaren Typendifferentialdiagnose zu gelangen. In Bestätigung der Literaturangaben wurde festgestellt, dass der VZ-Typ zum A-Typ, der Y-Typ zum D-Typ und der Z-Typ zum H-Typ gehören. Durch gekreuzte Absorptionsversuche wurden die Antigenformeln der verbleibenden 5 Typen ermittelt, die folgendermassen lauten:

Flexner A = 1, 2, 4, 5, 7
 » D = 1, 3, 5, 8
 » H = 1, 2, 4, 6
 » WX = 1, 3, 4, 6
 » W = 1, 4, 5, 7.

Zur praktischen Typendiagnose mit Hilfe der Objektglasagglutination eigneten sich 3 absorbierte Seren, nämlich ein 2-Serum (Serum A mit Kultur W absorbiert), ein 3,8-Serum (Serum D mit Kultur A absorbiert) und ein 5,8-Serum (Serum D mit Kultur WX absorbiert), die folgende Resultate ergaben:

Typ	Faktoren-Seren		
	2	3,8	5,8
A	++	—	+
D	—	++	++
H	++	—	—
WX	—	++	—
W	—	—	+

Es besteht kein Zweifel, dass — genau so wie in der *Salmonella*-Gruppe — nur die konsequente Durchführung *serologischer* Untersuchungen die Differentialdiagnose in der Flexner-Gruppe klarstellen kann. Mit Hilfe kultureller Methoden müssen dann die einzelnen Serotypen in verschiedene Vergärungstypen eingeteilt werden.

Es wird auf die Bedeutung der Serotypen für die *Widal*-Reaktion und die Herstellung von Vakzinen hingewiesen.

Tage Kemp: *Eine dimere erbliche Krankheit bei Mäusen* (mit Filmdemonstration).

Wird später in *Acta path. et microbiol.* publiziert werden.

ABSTRACTS — ANALYSES — REFERATE.

Dr. med. Paul Kallós: »Beiträge zur Immunbiologie der Tuberkulose« bei AB. Hasse W. Tullbergs Förlag, Stockholm 1941.

In seiner Monographie aus dem »Wenner-Gren Institut für experimentelle Biologie« der Universität Stockholm hat P. Kallós in klarer und übersichtlicher Weise eine Reihe von Betrachtungen und experimentellen Arbeiten zusammengestellt, die für die Immunbiologie der Tuberkulose von Bedeutung sind.

Nachdem einige Prinzipien der Immunitätslehre im einleitenden Kapitel erörtert worden sind, wird über »Stoffwechseluntersuchungen« an Bakterien (Hefe und *Salmonella enteritidis*), Erythrocyten und Gewebeschnitten unter Antikörperwirkung berichtet. Diese Versuche ergaben, »dass die Antikörper eine wesentliche Lebenstätigkeit, nämlich die Atmung, derjenigen Zellen (Mikroorganismen oder Körperzellen), gegen welche sie gerichtet sind, nicht zu schädigen imstande sind«. Im folgenden Kapitel werden Untersuchungen über die Einwirkung von homologem Antiserum auf die Vermehrung von Mikroorganismen in der Kultur mitgeteilt, und zwar unter Verwendung von Hefe, *Salmonella enteritidis* und Tuberkulosebazillen.

Das spezifische Antiserum vermochte die Vermehrung der betreffenden Mikroorganismen nicht zu hemmen. »Durch Zusammenwirken von Antikörpern und gewissen Zellelementen (Leukoeyten) scheint eine Hemmung der Vermehrung möglich zu sein«.

Der Verfasser geht dann auf den Hauptteil seines Buches, die Bedeutung der Immunitätsvorgänge bei der Tuberkulose, ein und beschreibt — nach einer Erörterung der cellulären Grundlagen der Immunitätsvorgänge — die Vermehrung der Tuberkulosebazillen im empfänglichen Tier (Meerschweinchen) und ihre Beziehungen zu den Reaktionsäusserungen des Wirtes. Es wird besonders auf die Bedeutung des Makrophagen-Systems bei der Abwehr einer tuberkulösen Infektion hingewiesen. Die Versuche erstreckten sich auf Meerschweinchen, die teils unvorbehandelt, teils mit Tuberkulose-

bazillen (durch Hitze abgetötet und in Paraffinöl aufgeschwemmt) immunisiert, einer Infektion mit lebenden Tuberkulosebazillen ausgesetzt wurden.

Ferner werden Untersuchungen über die Impftuberkulose der weissen Ratten mitgeteilt, bei denen keine natürliche Tuberkuloseimmunität besteht, sondern bei denen das eigentümliche Verhalten gegenüber der Tuberkuloseinfektion auf den Zustand ihres Makrophagen-Systems, das unter der Wirkung der latenten Infektion mit *Bartonella muris ratti* steht, zurückzuführen ist.

Es folgen dann »Studien über die Organwahl der Tuberkulose«, nach denen die Unempfänglichkeit der Meerschweincheniere für Tuberkulose durch Injektion eines Antinierenserums durchbrochen wird.

Es ist im Rahmen eines kurzen Referates nicht möglich, alle in Betracht kommenden und von *Kallós* erörterten Tuberkulose-Probleme zu diskutieren, so dass auf die eigene Lektüre dieses anregenden und wertvollen Buches hingewiesen werden muss.

F. Kauffmann.

GERHARD ARMAUER HANSEN.
THE DISCOVERER OF THE LEPRO BACILLUS
(1841—1912).



A century has passed since *Gerhard Armauer Hansen* was born on the twenty-ninth of July 1841. He succeeded early in demonstrating the specific cause of leprosy. Throughout life he continued ardently to explore leprosy problems. Moreover, he proved to be a clever hygienist who formulated and effectuated practical legislation for the control of the disease.

It was in 1847 that *D. C. Danielssen* and *C. W. Boeck* published their classical monograph »*Om Spedalskhed*« — On Leprosy — which was destined to play a fundamental rôle in the modern scientific investigation in leprosy. This work presented for the first time a clear description of the clinical and histo-pathological picture of leprosy. In regard to the etiology of the disease, this work maintained the hereditary transmissibility or hereditary dyscrasia of leprosy.

Armauer Hansen in 1868 became resident physician of Lungegaards Hospital at Bergen, where *Danielssen* was physician-in-chief. During this service he very soon became a fierce opponent of the *Danielssen-Boeck* hereditary diathesis hypothesis. Aided by histo-pathological leprosy investigations in and clinical observations of the course of the disease, *Armauer Hansen* became thoroughly convinced that leprosy is a contagious disease which necessarily must have a specific cause.

Bacteriology was then in its very beginning and the bacteriological technique was but little developed. However, *Armauer Hansen* succeeded in demonstrating rod-shaped bodies in leprous nodules. These microörganisms could be stained dark with osmic acid. In his 1874 publication, which appeared as a supplement to »*Norsk Magazin for Lægevidenskaben*«, he is exceedingly cautious. He concludes that he has encountered, in leprous nodules, bacteriform elements which possibly are the poison he presumes to be the cause of the disease.

When *Weigert* and *Koch* had discovered new methods for staining bacteria, *Armauer Hansen* attempted at once to stain his lepra bacilli by their methods. During these staining operations, *Neisser* arrived at Bergen in 1879 to investigate leprosy. *Armauer Hansen* demonstrated for him his findings in the hope that *Neisser*, who came directly from Breslau where *Weigert* and *Koch* had developed their staining methods, could possibly aid him. But the staining of his lepra bacilli failed also in the hands of *Neisser*. *Armauer Hansen* then presented *Neisser* with a great many sections of leprous material which this author took with him to Breslau. Here

Neisser succeeded in staining the lepra bacilli. Immediately he set about to publish an article on the specific bacterial cause of leprosy. In the meantime, *Armauer Hansen* also succeeded in staining his lepra bacillus after *Robert Koch* had advised him to prolong the staining process. This is the background of the earlier description of the lepra bacillus as the Hansen-*Neisser* bacillus. But in view of the fact that *Armauer Hansen* had discovered the lepra bacillus in 1873 — six years before *Neisser* — the name of the latter has subsequently been eliminated from the nomenclature of the *Mycobacterium leprae*. At the First International Lepra Congress at Berlin in 1897, *Armauer Hansen* was unanimously declared to be the discoverer of the lepra bacillus.

Armauer Hansen's discovery necessarily brought about a change not only in the conception of the cause of leprosy, but also in the manner in which the disease could be brought under effective control. In 1875 *Armauer Hansen* became physician-in-chief for the lepers in Norway, a position he held until his death. In this capacity he elaborated proposals for legislative reforms. In close agreement with the ideas he advocated with regard to the contagious nature of the disease, all subsequent legislation in behalf of the lepers in Norway has been evolved. The Norwegian leprosy law of 1877 and the re-inforced law of 1885, are the fruits of his indefatigable endeavours.

According to the latter law, lepers may be ordered by the health authorities to live in precautionary isolation from their families and immediate surroundings. Exceptions have been made for married couples who desire to live together. If the health authorities find that these regulations are not complied with, they may order the recalcitrant leper to be isolated in a state leprosy asylum, if necessary with the aid of the police. Thus the law of optional compulsory isolation permitted the leper to live at home provided he observed the precautionary regulations. In spite of this leniency, the law roused violent opposition, and it was even insinuated that the leper law placed the diseased in a class with criminal con-

victs. However, in view of the excellent results derived from these precautionary health regulations, the opposition against the law gradually ceased entirely. The following statistics illustrate the rapid decrease of leprosy in Norway:

<i>Year</i>	<i>Number of lepers</i>
1875	1752
1885	1195
1895	688
1900	577
1910	326
1920	160
1930	69
1941	26

Owing to his elucidation of the nature and of the specific cause of leprosy, *Armauer Hansen's* endeavours to gain control of the disease, that terrible and justly dreaded affection which, in his youth, was widely spread throughout Norway, now, a century after his birth, is nearly stamped out.

The Norwegian leprosy law has served as a model for leprosy legislation in many other countries. Its influence has asserted itself wherever attempts were made at suppressing the disease.

Armauer Hansen received numerous proofs of appreciation. On the occasion of his sixtieth birthday, in 1901, his portrait bust, to which colleagues and friends in several countries had contributed, was unveiled in the gardens of the Bergen Museum. In 1909 *Armauer Hansen* presided over the Second International Leprosy Congress held at Bergen. He was made honorary doctor by the University of Copenhagen, and honorary or corresponding member of a series of scientific societies.

Armauer Hansen died on February 12, 1912, during a professional journey to Florö near Bergen. An active and meritorious life devoted to science for the benefit of humanity thus came to an end.

Th. M. Vogelsang.

ON THE NATURE OF GONADOTROPHIN IN CASES OF MALIGNANT TUMORS OF THE TESTIS.*)

By *Christian Hamburger.*

(Received for publication December 22nd, 1940).

In the last decade, gonadotrophin determinations on the urine have been adopted as a routine method of examination in cases of tumors of the testis, and there can be no doubt that demonstration of gonadotrophin in the urine may be of great significance to the diagnosis as well as to the prognosis. Unfortunately, most investigators have paid attention merely to a quantitative determination of the gonadotrophins, without making any attempt to estimate the particular nature of the hormone.

With our present knowledge of their biology, the gonadotrophins may be divided into three main types:

- 1) *Chorionic gonadotrophin*, the gonadotrophic hormone from the urine, blood and placenta of pregnant women. International standard preparation available.
- 2) *Gonadotrophin from pregnant mares' serum*, from the blood and endometrium of pregnant mares. An international standard preparation of this hormone is available too.

*) Lovens kemiske Fabrik, Copenhagen, has generously placed large amounts of Physex, Antex and Follicle-stimulating Hormone at my disposal for the studies here presented.

3) *Hypophysial gonadotrophin*, which is hardly one hormone, but probably composed of a follicle-stimulating (gametokinetic) factor and a luteinizing (interstitial cell-stimulating) factor. In the absence of gonadic function, man and several animals excrete with the urine a hormone that is to be considered identical with the follicle-stimulating factor from the hypophysis.

The significance of *qualitative* gonadotrophin determination in cases of malignant tumor of the testis has been emphasized in particular by Bang, Hamburger & Jens Nielsen (1933—1939)^{1,2,11,12,14,17}) whose studies showed that some testicular tumors — the X-ray-resistant »mixed epithelioma«, often containing syncytial cell elements — produce themselves a gonadotrophin that is identical with the chorionic gonadotrophin of the pregnant female organism, while other testicular tumors (especially seminomata, which, as a rule, are sensitive to X-ray treatment) are not hormone-producing by themselves, whereas for some reason, still obscure, they give rise to excretion of hypophysial gonadotrophin, just like in castrates.

So far only a few occasional reports have been given of studies aimed especially at the question about the nature of the gonadotrophin in malignant testicular tumors. Gerber (1933)¹⁰) and Zondek (1935)¹⁹) and others, it is true, have pointed out the fact that in some testicular tumors only follicle-stimulating hormone is excreted, while in others there is also an excretion of luteinizing hormone, but the gonadotrophin, has not been analyzed further.

Evans *et al.* (1933)⁶) examined the urine in a case of embryonal carcinoma of the testis. The urine contained 50,000 M. U. of gonadotrophin per liter, and this gonadotrophin was found to be of a particular type, as in some respects it behaved like chorionic gonadotrophin, in others like hypophysial gonadotrophin. Two other cases with up to one million units of gonadotrophin per liter of urine gave a similar mixed reaction (Evans & Simpson, 1935)⁴), but here this feature was found to be of quantitative nature, as also »pure« chorionic gonadotrophin gave the same reaction

when administered in equally large doses (Evans, 1936)³⁾ so that in these cases the gonadotrophin had to be looked as identical with chorionic gonadotrophin.

Main & Leonard (1934)¹⁰⁾ analyzed the urine of a man with teratoma testis and found — as it seemed, at that time — a peculiar gonadotrophin which now with a fair degree of certainty may be identified with the hypophysial gonadotrophin in castrate urine. Fluhmann & Hoffmann (1934)⁸⁾ were able after three different methods of examination to characterize the gonadotrophin in urine from a patient with teratoma testis as being chorionic gonadotrophin. A similar case has been reported by Freed & Coppock (1935).⁹⁾ In their first paper on hormonal analyses on 33 patients with malignant testicular tumors, Hinman & Powell (1935)¹⁵⁾ laid particular stress upon the biological nature of the hormone, but in a subsequent work by Powell (1938)¹⁸⁾ the entirely quantitative gonadotrophin determination was made the basis for a classification of the testicular tumors. Finally, Fevold, Fiske & Nathanson (1939)⁷⁾ examined the urine of a man with a chorione epithelioma of the testis. The hormone, of which the urine contained about 1 million R. U. per liter, was identical with chorionic gonadotrophin (tested on normal female and male rats, on hypophysectomized female rats, and on pigeons).

As only a few cases have been reported so far, an account will be given here of several cases of malignant testicular tumors in which the examination was aimed especially at a further analysis of the nature of the gonadotrophin. From our material, which now comprises more than 150 cases of testicular tumors in which hormonal analysis, I shall pick out preferably the cases for which complete dose-response curves have been obtained on infantile female rats.

1. Cases with Excretion of Chorionic Gonadotrophin.

Chorionic gonadotrophin has in itself merely a limited effect on the ovaries, but in connection with hypophysial

gonadotrophin it produces — especially in rodents — maturing of follicles (with secondary oestrous changes) and corpus luteum formation; but these effects do not appear in hypophysectomized animals. In normal infantile female rats the smallest effective doses give oestrous changes in the vagina (cornification) and enlargement of the uterus. On administration of somewhat larger doses, mature follicles appear in the ovaries; and with a greater dosage several of these follicles are transformed into corpora lutea. Still, the number of stimulated follicles is rather limited (in contrast to the results obtained with mare serum and hypophysial gonadotrophin), and the weight of the ovaries is increased but little. On graphical plotting of the ovarian weight increase the curve is seen to reach a plateau rather rapidly, and for a good many years it was an established dogma that chorionic gonadotrophin was not able to stimulate the ovarian weight to more than 3—4 times the ovarian weight of the control animals. But, Evans & Simpson (1935)⁴) and Evans (1936)⁵) showed that when rats were given about 10,000 times the minimal effective dose, the ovarian weight rose rather abruptly (see Fig. 1). This form of reaction curve for chorionic gonadotrophin is confirmed by the experiments reported below:

1) A *highly purified chorionic gonadotrophin preparation*, »Physex 1938«, with about 63,000 I. U. per gram (diluted with lactose). Dose-response curves were worked out for the uterine and ovarian weights in rats, 26—28 days old. In this and the following experiments the dose was divided into 5 equal injections given in 48 hours, and autopsy was performed about 100 hours after the first injection. The ovaries and the emptied uterus were weighed in fresh condition. The lowest examined dose of »Physex 1938« was 0.00078125 mg.; then the dose was doubled continually to the highest dose, 204.8 mg. Thus the greatest dose was about 256,000 times larger than the smallest. The outcome is seen in Fig. 2, where each point on the curve represents the average result from 5—8 rats (a total of 130 rats were employed for this curve).

CHART I.

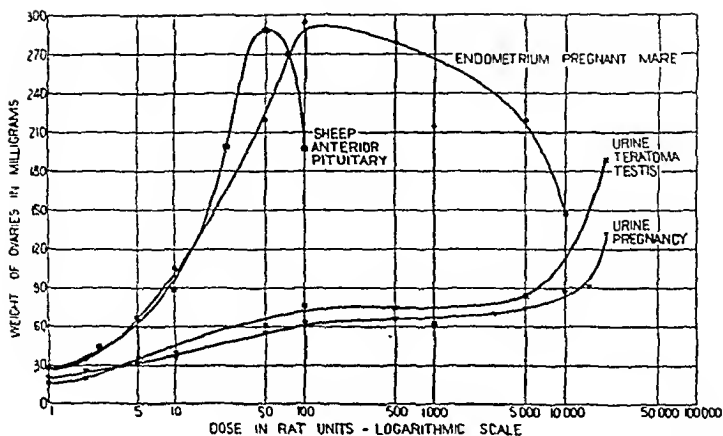


Fig. 1.

Dose-response curves for different gonadotrophins. The two lower show the form of the ovarian weight curves for chorionic gonadotrophin. (After Evans, (1936³)).

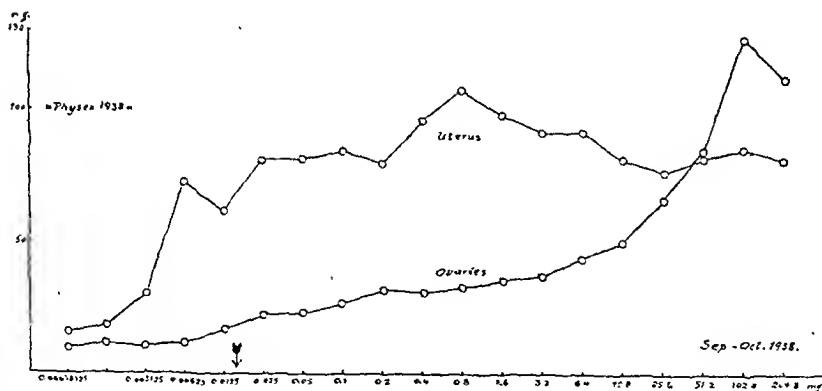


Fig. 2.

Dose-response curves for uterine and ovarian weights in infantile rats treated with the chorionic gonadotrophin »Physex 1938«. (The average weight of the organs is plotted along the axis of ordinates, the total dose in logarithmic scale along the axis of abscissas. The arrow marks the place for 1 international unit of chorionic gonadotrophin).

2) *Chorionic gonadotrophin preparation, »Physex 39038«,* with about 20,000 R. U. per gram (diluted with lactose). This preparation was tested in doses from 1/200 mg. to 100 mg. with rather large intervals in the dosage; 4—5 rats were used for each dose, altogether 32 rats. The curves are shown in Fig. 3.

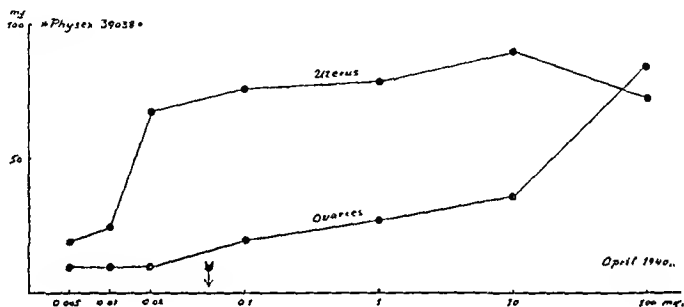


Fig. 3.

Dose-response curves for uterine and ovarian weights in infantile rats treated with the chorionic gonadotrophin »Physex 39038«. (For additional explanation, see the legend of Fig. 2).

3) *Urine of a patient with metastases of mixed epithelioma of the testis.*

Case Record: P.U.D., aged 28, with a past history of good health. In August 1938, pain in the left testis, which felt enlarged. No treatment for 6 weeks, during which period the testis did not increase in size. Adm. to Dep. A., Bispebjerg Hospital. 8/10—38: *Hemicastration*, on the left side. The testis consisted of a walnut-sized tumor, which on section was greyish-yellow in colour, with small nodular areas in the tissue and small hæmorrhages. *Microscopic diagnosis:* *Mixed epithelioma of the testis* (F. Bang). *X-ray treatment* in the Radium Station, Copenhagen, from 3/11—38 to 19/1—39 (at first ambulatory, then hospitalization from 28/12) for *abdominal metastases*; these were growing rapidly, and, after increasing cachexia, the patient died on 31/1—39, that is, 5—6 months after the primary tumor was noticed the first time. There was *no gynækomasty*. — *Autopsy* revealed extensive metastases to the liver, lungs and lymph glands. Microscopy of the liver and lung metastases: Many areas of necrosis and hæmorrhage; broad streaks of vigorous fibrous stroma, between which there are epithelial cells with large, somewhat vesicular nuclei, often with nucleoli, and

slightly basophile cytoplasm, as a rule without any distinct cell membrane. The cells formed compact heaps, here and there with an admixture of, or transition to, *syncytial cells of trophoblast type* with dark close-packed nuclei. *Microscopic diagnosis: Metastases of mixed epithelioma with transition to chorionepithelioma* (F. Bang), see Fig. 4. The hypophysis contained an abundance of eosinophile cells.



Fig. 4.

Microphoto of metastasis of mixed epithelioma of the testis (Case P. U. D.). Note the syncytial cells. Magnif. $\times 75$.

Hormonal analyses: The excretion of chorionic gonadotrophin increased from about 3,000 I. U. per day, shortly after the hemicastration, to about 300,000 I. U. per day, shortly before death. In the later specimens the excretion of oestrin was increased (> 500 , < 1000 M. U. per day; normally in men: > 20 , < 50 M. U. per day). The adrogenic hormone content of the urine was very low (< 1 I. U. per day). No gonadotrophin could be demonstrated in the hypophysis by implantation of 8—10 mg. of this organ in infantile female mice; implantation of similar fragments of normal hypophysis produces a very strong gonadotrophin reaction.

From *liver metastases* a pyridin extract was prepared that contained chorionic gonadotrophin in an amount corresponding to from 300—1500 I. U. per g. of tumor tissue.

Two 24-hour urines (27/1 and 28/1—39) were pooled, and the mixture was tested on infantile female rats in 15 different doses from 1/3200 to 8 cc. of untreated urine, with 6—7 rats per dose, altogether 100 rats. The ovarian and uterine weight curves are shown in Fig. 5.

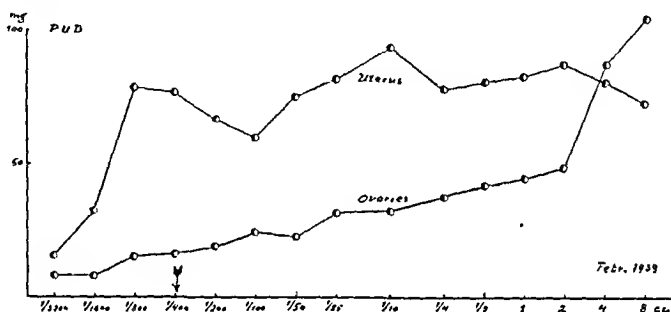


Fig. 5.

Dose-response curves for uterine and ovarian weights in infantile rats treated with native urine from Case P.U.D. (For additional explanation, see the legend of Fig. 2).

In addition, examination of the vaginal smears from the rats, the corpus luteum formation in the mice (110 mice) and the reaction of the rabbits (28 animals) showed complete conformity with the curves for the international standard preparation for chorionic gonadotrophin (cf. Hamburger, 1939).¹³⁾

4) Urine of a patient with metastasizing mixed epithelioma of the testis.

Case Record: L. G. S. aged 24, with a past history of good health. A tumor of the left testis was noticed in May 1939. In Dep. D, Bispebjerg Hospital, on 20/6—39, the tumor (size of a goose egg) was removed. *Microscopic diagnosis:* Mixed epithelioma of the testis (F. Bang). 3/7—39: Admitted to the Radium Station for X-ray treatment of metastases. On admission, his general condition was good; there was some *mammary hypertrophy* (later on, a secretion could be expressed). Roentgenography showed metastases to the lungs,

and on palpation a doubtful resistance could be felt deeply in the left side of the abdomen. During this and subsequent stays in the Radium Station the abdominal tumors were noticed to grow very rapidly (4/11—39: more than head-sized tumor in the epigastrium; in the left flank, a coconut-sized tumor, etc.). There were dyspnoea and cyanosis; later, jaundice, severe abdominal pain, and rapidly progressing cachexia. The patient died on 21/11—39, about six months after the discovery of the testicular tumor. — *Autopsy* revealed ex-



Fig. 6.

Microphoto of metastasis of the adenocarcinoma type, following mixed epithelioma of the testis (Cace L. G. S.). Magnif. $\times 75$.

tensive metastases to the liver, lungs and lymph glands. Microscopy of the metastases: Extensive necrosis of the tumor tissue, which is built up chiefly of scattered epithelial cells with large, angular, often vesicular nuclei, and between these cells there are glandlike tubular structure lined with similar epithelium, which in some places is slightly columnar. *Microscopic diagnosis: Adenocarcinoma type* (F. Bang). Microscopy of the right testis showed moderate atrophy of the testicular canals. *The anterior lobe of the hypophysis* consisted chiefly of eosinophile cells.

Hormonal analyses: As in the preceding case, there was an enormous increase in the excretion of chorionic gonadotrophin, from a few thousand I. U. per day at the time of

hemicastration (and shortly after) to about 80,000 I. U. per day in the following months. The oestrin excretion was somewhat increased (100—200 M. U. per day). The androgenic hormone content of the urine was somewhat decreased (10—20 I. U. per day); and shortly before death it was only about 2 I. U. per day.

From 8 24-hour urines (27/10 to 4/11) a dry powder was obtained by tannic acid precipitation (weight of the entire powder: 5.0 grams). The dose-response curves for uterine and ovarian weights in infantile rats (see Fig. 7) covered

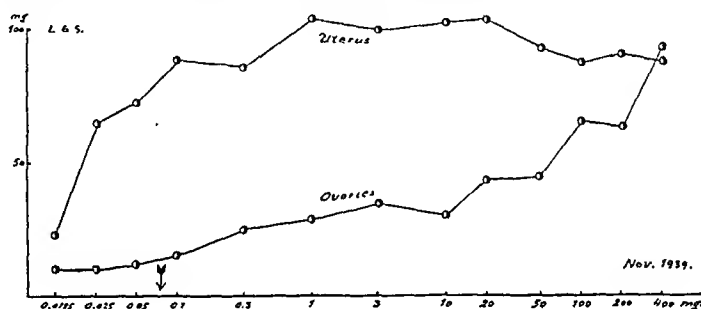


Fig. 7.

Dose-response curves for uterine and ovarian weights in infantile rats treated with tannate of urine from Case L. G. S. (For additional explanation see the legend of Fig. 2).

the doses from 1/80 mg. to 400 mg. As the powder was hard to dissolve, it was somewhat difficult to give the doses 200 and 400 mg. 4—6 rats were employed per dose, altogether 57 rats.

5) Urine of a patient with mixed epithelioma of the testis + small-cystic mixed tumor.

Case-Record: E. H. S. aged 25, with past history of good health. 28/9—39, admitted to the Surgical Dep., Holbæk County Hospital. About 1 month before, onset of pain in the lumbar spinal column and enlargement of the left testis (size of duck egg) which only inconvenienced the patient by its size. 25/10-39: removal of the testicular tumor, which now had the size of a swan egg. Palpation of the abdomen was difficult. Roentgenography showed metastases to the lungs.

The tumor measured 7×13 cm. It was divided into two apparently quite separate tumors, the upper of which was somewhat gelatinous, with numerous fibrous streaks, and built up of small cysts (up to nut-kernel size) with a semifluid or gelatinous content. The lower tumor was surrounded by a fibrous capsule; it consisted in part of a rather large cavity containing yellowish necrotic debris, partly of a walnut-sized, projecting, red tumor area with a few

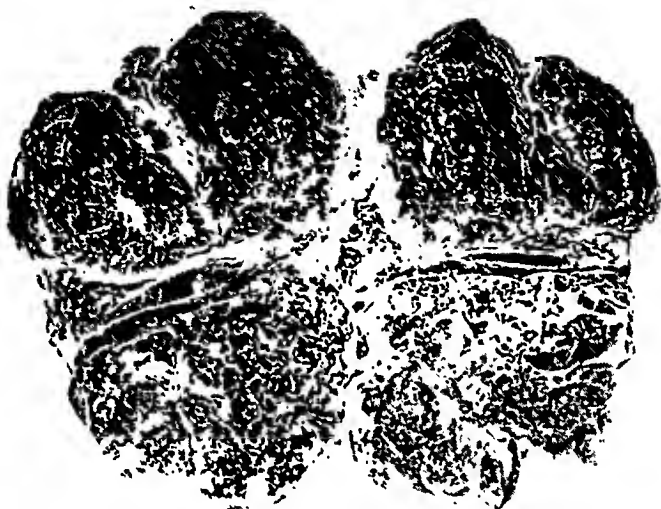


Fig. 8.

Photo of the testicular tumor in Case E. H. S. ($\frac{1}{2}$ natural size). The small cystic area is seen in the upper part of the testis; the lower part is made up of the placenta-like tumor (mixed epithelioma).

large veins, quite loose in consistency, resembling strikingly placental tissue (see Fig. 8). *Microscopic examination:* The upper tumor was built like a benign small-cystic mixed tumor with small organoid cysts, islands of cartilage and streaks of smooth muscle. The placentalike tumor consisted of papillomatous tumor tissue with large thinwalled capillaries in the axis of the papillae. Microscopically there was no resemblance to placental tissue; syncytial cells and Langhans cells could not be demonstrated — nor seminoma or chorionepithelioma tissue. The epithelium consisted of one or more layers of cells which most often were cuboidal, but sometimes columnar; there was a marked tendency to necrosis. The cells were of quite the same character as those encountered in mixed epithelioma

(often designated as adenocarcinoma). In one area the placenta-like tumor was seen to invade the fibrous capsule without reaching into the tumor tissue in the cystic tumor. *Microscopic diagnosis: Small-cystic mixed tumor, histologically benign; Mixed epithelioma of the testis.* (F. Bang).

13 days after the operation, thrombosis appeared in the left lower extremity, and a few days later in the right leg. The patient died on 22/10—39, 17 days after the hemicastration. *No autopsy.*

Hormonal analyses. The urine was examined twice: 1) on 30/9—39 (before the hemicastration), when it contained about 100,000 I. U. of chorionic gonadotrophin per day, about 20 M. U. of oestrin and 29 I. U. of androgenic hormone per day. 2) on 5/10—39 (on the day of the operation), when it showed 64,000 I. U. of chorionic gonadotrophin, about 40 M. U. of oestrin and 25 I. U. of androgenic hormone per day.

The *primary tumor* was divided into two parts: the small cystic part and the mixed epithelioma part. From each of these parts there was prepared a saline emulsion that contained respectively 40 and 50 I. U. of chorionic gonadotrophin per gram of tumor tissue, and also a pyridin extract which showed respectively 61 and 62 I. U. per gram of tumor tissue. So, notwithstanding their different histological structure, the two parts of the tumor contained about the same amount of gonadotrophin, probably because the blood, lymph and every tissue were »saturated« with the hormone. Other cases in which tumor tissue, muscle and other tissues were analysed at the same time, showed about the same amount of hormone in the various tissues — something which naturally detracts considerably from the value of the tissue analyses. The gonadotrophin of the tumor extracts from this patient gave dose-response curves of the same type as obtained with chorionic gonadotrophin.

The 24-hour urine from 30/9—39 was assayed on infantile rats in doses from 1/800 cc. to 8 cc. of untreated urine (greater doses were toxic to the animals). 5—10 rats were used per dose, 86 rats altogether. The uterine and ovarian weight curves are shown in Fig. 9.

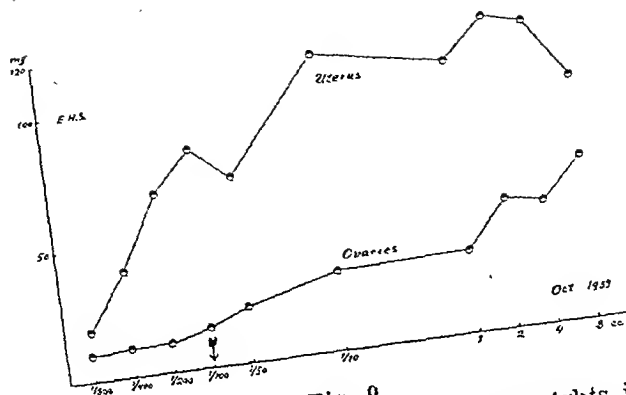


Fig. 9.

Dose-response curves for uterine and ovarian weights in infantile rats treated with native urine from Case E. H. S. (For additional explanation, see the legend of Fig. 2).

6) Urine of a male patient with extragenital(?) chorion-epithelioma.

*Case Record**): O. C. H., male, aged 38, with a past history of good health. 4 months prior to admission, onset of pain in the lumbar region, especially on the left side. During this period the mammae have become somewhat enlarged. There was some loss of weight. Otherwise no symptoms. On 15/6—39 admitted to Dep. V (Surgery), the Kommunehospital. On admission, the patient was pale, somewhat emaciated and distressed with pain. Laterally under the left costal margin a firm tumor could be felt. There was some bilateral *mammary hypertrophy*. Both testes appeared to be normal. 7/7—39: Partial extirpation of the tumor in the kidney region. The patient died on the following day. *Autopsy* showed a tumor, as large as the head of a man, in the left kidney region, strongly adherent to the surroundings and invading the ileo-psoas, besides metastases to the liver and lungs. The left testis was a little smaller than the right; in its center there was a small pale and soft area. *Microscopic diagnosis of the tumor in the kidney region and the metastases*: Chorionepithelioma. The suspect area in the left testis was found to consist of fibrous tissue with many Leydig cells (Sv. Petri). The case had to be interpreted as a primary extragenital chorionic epithelioma, or metastases of chorionic epithelioma from a minimal focus in the left testis.

*) This case has been described separately by Heiberg & Hamburger (Nord. Med. 9 — 141 — 1941).

Hormonal analyses: The urine was assayed 3 times in the course of 10 days. It showed up to 1 million I. U. of chorionic gonadotrophin per liter (600,000 I. U. per day), 800—1000 M. U. of oestrin, and normal values for androgenic hormon: 32 and 44 I. U. The *tumor tissue* (removed by operation) contained about 2500 I. U. of chorionic gonadotrophin per gram tumor tissue (pyridin extract).

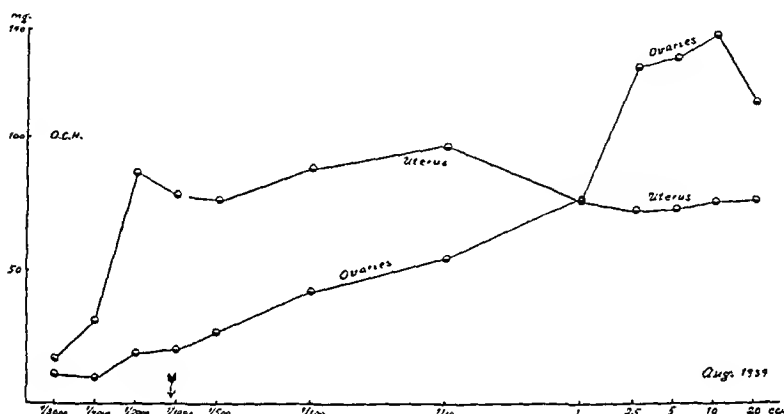


Fig. 10.

Dose-response curves for uterine and ovarian weights in infantile rats treated with native urine from Case O. C. H. (For additional explanation, see the legend of Fig. 2).

One of the 24-hour urines was tested on 81 infantile female rats in doses from 1/8000 cc. to 20 cc. of untreated urine; on an average, 7 rats were employed per dose. The uterine and ovarian weight curves are shown in Fig. 10. In addition, the urine was tested on 31 infantile female mice and 15 rabbits. The reaction curves from these experiments were likewise found to be identical with the curves for the international standard preparation for chorionic gonadotrophin.

Comparison between the six curves presented here shows an excellent agreement, in particular when it is taken into consideration that they are carried out at different times

within a period of $1\frac{1}{2}$ years. In Fig. 11 all the curves are presented in the same diagram. It will be noticed how close they all come to the average chorionic gonadotrophin curve. In the cases where microscopic examination is made of the ovaries there is likewise a complete agreement between the gonadotrophin content of the analysed urines and the assay of chorionic gonadotrophin.

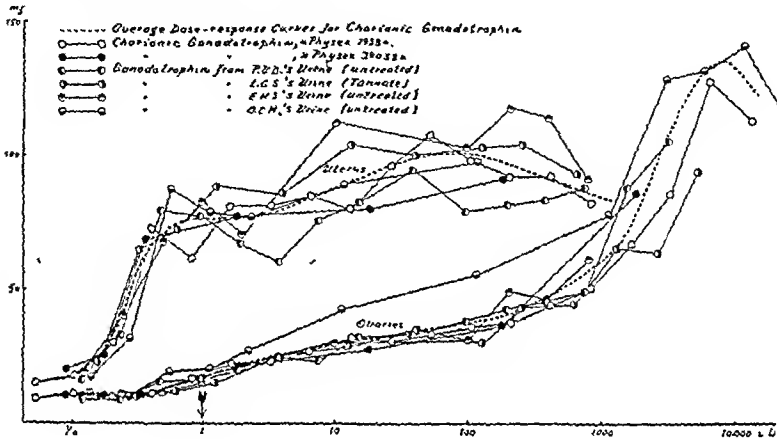


Fig. 11.

The curves from Figs. 2, 3, 5, 7, 9 and 10 recorded in the same diagram. The uterine weight rises abruptly between about $\frac{1}{8}$ and $\frac{1}{2}$ I. U., and then it reaches a plateau. (For the sake of easy survey, the uterine weights obtained with the largest doses are not recorded in this diagram). The ovarian weight rises gradually from about $\frac{1}{2}$ I. U. to about 1000 I. U., whereafter there is a rather abrupt rise till the maximum is reached, at about 10,000 units.

The rather abrupt rise appearing in response to the doses from about 1,000 to about 10,000 I. U., has given rise to various considerations. One might imagine that the rise was not brought about by the chorionic gonadotrophin but by hypophyseal gonadotrophin, small amounts of which are claimed to be present in every urine, and that this substance would exert its influence with the high doses. There are several features, however, that go against this hypothesis: In all the cases here examined the rise appeared in about the same

place in the curve, although the gonadotrophin was derived from such different sources as highly purified preparations from the urine of pregnant women, a crude tannate, and several native urines of patients with various testicular tumors, besides from a chorionepithelioma in a man. If the mentioned hypothesis were correct there would in all these cases have been approximately the same proportion between the chorionic gonadotrophin and the possibly present hypophysial gonadotrophin, which would be a quite unreasonable assumption. Indeed Fevold et al. (1939)⁷⁾ arrived at the result that in their aforementioned case the active substance consisted entirely in chorionic gonadotrophin, as the gonadotrophin in their case was not attenuated by treatment with tricresol — something, they claim, that happens to hypophysial gonadotrophin (in castrate urine). However this may be, there can be no doubt that *the gonadotrophin demonstrated in the urine in the present analyzed cases of malignant testicular tumors is identical with the chorionic gonadotrophin in pregnant women.*

II. Excretion of Follicle-stimulating Hypophysial Gonadotrophin.

When the normal function of the ovaries or testis is lost (castration, climacterium), the anterior pituitary lobe undergoes certain secretory changes, the production and yield of the follicle-stimulating factor being increased. This factor can be demonstrated in the blood and urine, but the amount of hormone excreted with the urine is relatively small, up to about 400 M. U. per day, and the excretion is subject to wide variation from day to day. Still, apart from these variations, the excretion is permanent. In order to demonstrate the presence of this hormone in the urine, it is necessary in most cases to precipitate the hormone (with alcohol or tannic acid). Its action in the organism consists in stimulation of the germinating tissue (follicular apparatus and seminiferous

tubules). In the ovaries there is produced a general ripening of the follicles, while corpus luteum formation appeared only in response to large doses, and it is due in part to the activity of the animal's own hypophysis. The form of the dose-response curves for ovarian and uterine weights in infant-

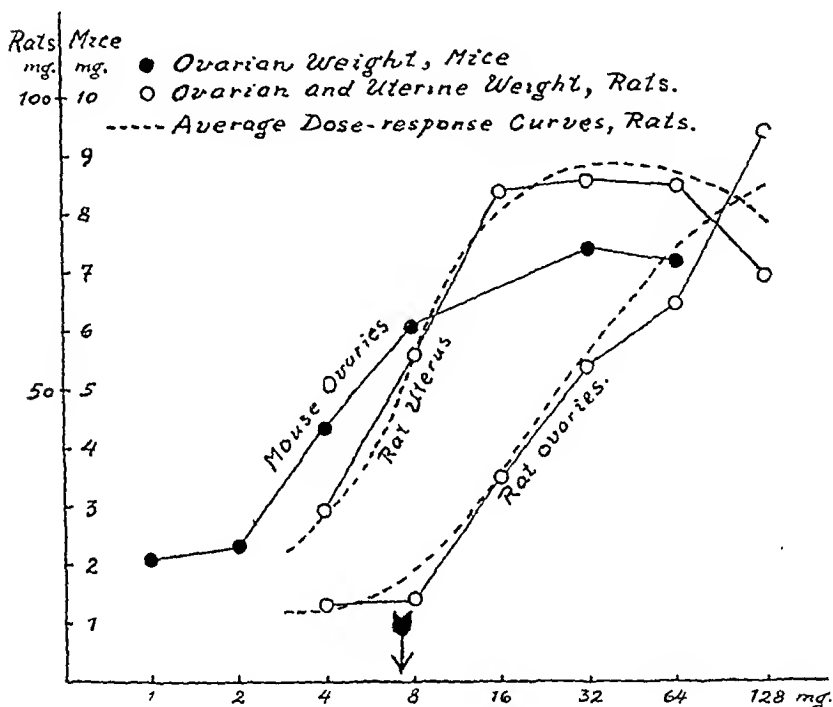


Fig. 12.

Dose-response curves for ovarian weight in infantile mice, and for ovarian and uterine weights in infantile rats, after treatment with a tannate of pooled menopausal urines (hypophysial follicle-stimulating gonadotrophin). The ovarian weight increases abruptly already before the maximal uterine effect is reached. The arrow indicates the place for 1 R. U. (uterus 50 mg.).

tile rats and mice differs widely from the corresponding curves for chorionic gonadotrophin (see Fig. 12). From the rat curves it is evident that the ovarian weight increases greatly even on administration of relatively small doses. The

qualitative estimation of the follicle-stimulating hypophysial gonadotrophin is carried out most easily by microscopic examination of the ovaries in infantile mice. The changes appearing here are so characteristic that the diagnosis is difficult only when the assay concerns only a quite minimal hormonal effect. In sections the ovaries are seen to be studded with numerous, middle-sized, Graafian follicles (for particulars, the reader is referred to Hamburger (1933).¹¹)

Follicle-stimulating hormone is present, in demonstrable amounts — *i. e.* ≥ 50 M. U. per day — in the urine in about 75 % of the patients with *seminoma of the testis* or with *small-cystic mixed tumors*. It may be found before the primary tumor is removed, and before the patient has received X-ray treatment; but the excretion of this hormone does not cease after the removal of the primary tumor, and it is quite independent of the development or absence of metastases. It has been difficult to explain the occurrence of this hormone in the patients with testicular tumors, but after it has been shown, for one thing, that the excretion of *androgenic hormones* in these cases are down at the level of the excretion in castrates (Hamburger & Godtfredsen, this journal p. 485) it seems more likely that the excretion of follicle-stimulating hormones means a *decrease in the functional capacity of the testis*. In some cases, testicular tumor patients have been excreting follicle-stimulating hormone for a while, and later this hormone has given way to chorionic gonadotrophin — namely, at the point of time when metastases of the mixed epithelioma type had developed. Our entire material includes but two cases in which there was a demonstrable *simultaneous excretion* of chorionic gonadotrophin and hypophysial gonadotrophin (follicle-stimulating hormone). One of these cases has been reported previously (Hamburger, 1933,¹¹) *l. c.* p. 157—164); the other, far better investigated, will be reported in the following section.

III. A Case of Testicular Tumor with Simultaneous Presence of Chorionic and Hypophyseal Gonadotrophin in the Urine.

Case Record: P. E. N., aged 25, with past history of good health. About half a year before hospitalization, onset of pain in the right testis; a few months after the testis commenced to increase rapidly in size. In August 1938 the testicular tumor was removed, in the Roskilde County Hospital. *Microscopic diagnosis: Small-cystic mixed tumor (histologically malignant) with embryonal-like structure and syncytial trophoblast-like cells.* (F. Bang). 29/8—38 the patient was admitted, the first time, to the Radium Station, Copenhagen. There was no evidence of metastases. Prophylactic X-ray treatment was given (irradiation of the abdominal lymph glands). There was no gynæcomasty. During the following year the patient was admitted several times to the Radium Station for X-ray treatment. In May 1939, 9 months after the hemicastration, examination revealed in the epigastrium, one finger's breadth above the umbilicus, the presence of an intra-abdominal roundish tumor (size of a green walnut), rather close to the anterior abdominal wall; it was fairly movable and somewhat tender. 28/6: *explorative laparotomy* (in the Surg. Dep. of the Finsen Institute). Several mesenteric intumescences were palpable, and one of them was removed for microscopy. (*Microscopic diagnosis: Lymph gland inflammatory reaction*). During the following months the abdominal tumors were increasing in size; the patient was getting worse rapidly, troubled with pain, nausea and vomiting. In October 1939 there was an intumescence (size of a child's head) in the depth of the abdomen, at the level of the umbilicus, and signs of enlargement of the deep lymph glands in the right iliac fossa. The left testis was a little undersized. The patient was given palliative irradiation of the metastases, on which the abdominal discomfort subsided somewhat. He was discharged on 16/11—39, and died at home 23/12—39 (*i. e.* 16 months after the hemicastration) with metastases to the lungs, mediastinum and lumbar lymph glands. *No autopsy.*

Hormonal analyses: The urine was not analyzed until shortly after the hemicastration; no increase could be demonstrated in the gonadotrophin content of the urine. But, in the second half-year after the removal of the primary tumor, 4 examined 24-hour urines showed about 500 M. U. of follicle-stimulating hormone per day. Several analyses presented the peculiar phenomenon that, corpora lutea had developed in several of the mouse ovaries — something that

might conceivably be connected with the great hormonal content. A specimen of urine from 29/7—39 (see Fig. 13) produced a quite typical reaction to follicle-stimulating hormone. During October—November 1939 (at a point of time when

----- *Average Dose-response
Curves for Follicle-stimulating
Gonadotrophin from
Castrate Urine.*

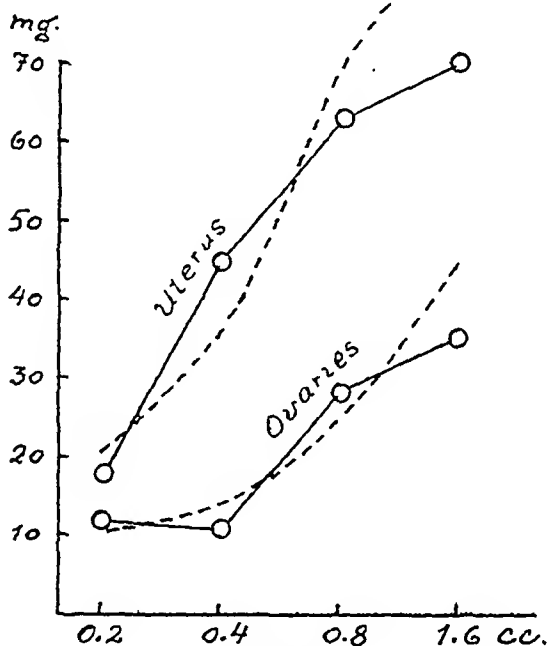


Fig. 13.

Dose-response curves for uterine and ovarian weights in infantile rats treated with native urine from Case P. E. N., July 1939. The form of the curves is typical of hypophysial follicle-stimulating gonadotrophin.

abdominal metastases had appeared) the urine was analyzed many times. The oestrin secretion was normal (> 20 , < 50 M. U. per day), the androgenic hormone excretion somewhat decreased (5 and 10 I. U. per day).

From this point of time the gonadotrophin content of the urine was examined with particular thoroughness on rats, mice and guinea pigs. In their form the uterine and ovarian weight curves for infantile rats and mice deviated from the chorionic gonadotrophin curves as well as from the follicle-stimulating hormone curves. On the other hand, these curves were found to show *complete concordance with the corresponding curves for mare serum gonadotrophin* (see Fig. 14, in which the dotted lines represent the average curves for numerous mare serum gonadotrophin preparations tested on more than 1,000 rats and 650 mice).

Also the histological picture of the mouse and rat ovaries was quite in conformity with the effect of mares' serum gonadotrophin; and this was the case with all the urines from the patient during this period. So it might look as if the tumor tissue of the patient produced the gonadotrophin which otherwise is found only in pregnant mares. But if it really were a hormone identical with mares' serum gonadotrophin, its secretion with the urine would indeed be very strange. For in the mare the hormone does not pass through the kidneys; nor is it excreted with the urine after its injection into monkeys and rats (Evans *et al*, 1933)⁵) — in contrast to chorionic and hypophysial gonadotrophin.

With a view to this feature, the following experiments were carried out on the *excretion of different gonadotrophins in rabbits*: 5 adult female rabbits, weighing between 2.020 and 2.170 kg. were given one injection of gonadotrophin immediately after the bladder had been emptied by manual expression. Then the urine was collected for 24 hours, the bladder being emptied again at the end of the experiment. The gonadotrophin content of the urine was determined by titration on infantile female rats. The results are given in Table 1. It is to be mentioned that all the doses are converted to rat units (1 R. U. = the dose of gonadotrophin able to produce an average uterine weight of 50 mg.) so as to obtain some means of comparison between the potency of the different gonadotrophins.

It is to be emphasized that none of these gonadotrophin preparations had undergone any qualitative change by passing through the rabbit organism. As shown in Table 1, of the injected chorionic and human hypophysial gonadotrophins 12.5 % was excreted in the first 24 hours; of a minor dose

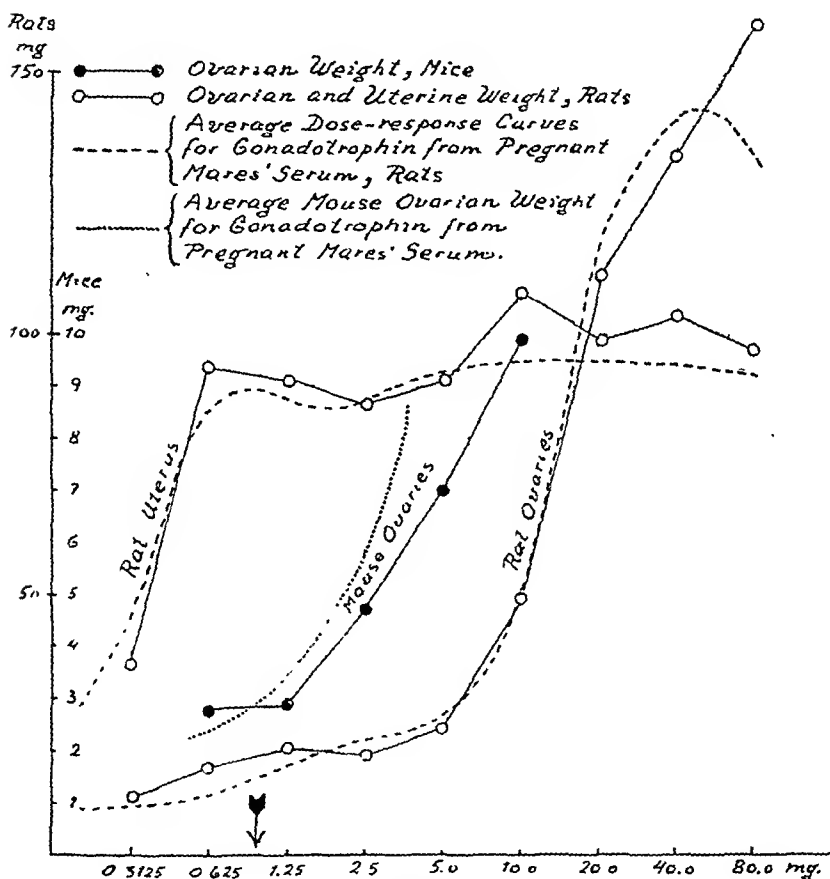


Fig. 14.

Dose-response curves for ovarian weight in infantile mice, and for uterine and ovarian weights in infantile rats, treated with a tannate of the urine from Case P. E. N., November 1939. The almost improbable concordance between these curves and the corresponding curves for the gonadotrophin from pregnant mares' serum is illustrated plainly by the graph. (The arrow indicates the place for 1 I. U. of mares' serum gonadotrophin).

Table 1.

Excretion of Gonadotrophin Injected into Normal Adult Female Rabbits.

Preparation	Dose	Excretion in 24 hours	
	inj. R. U.	abs. R. U.	%
Chorionic gonadotrophin (»Physex«)	320	40	12.5
Human hypophysial gonadotrophin*)	4000	500	12.5
Mares' serum gonadotrophin (»Antex«)	4000	62	1.6
» » » »	300	0 (<5)	0 (<1.7)
Gonadotrophin from Pt. P. E. N.**)	300	60	20.0

of mares' serum gonadotrophin no demonstrable amount was excreted, of a large dose only 1.6 % was excreted. On the other hand, no less than 20 % of the injected gonadotrophin from the patient (P. E. N.) could be demonstrated in the first 24-hour urine. *As to excretion, then, this hormone behaved like chorionic and hypophysial gonadotrophins*, differing in this respect from mares' serum gonadotrophin.

Quite the same result was obtained in experiments on *guinea-pigs*, in which the hormone gave the same reaction as did hypophysial and chorionic gonadotrophin, but differed from mares' serum gonadotrophin (these guinea-pig experiments, which were carried out in collaboration with K. Pedersen-Bjergaard, are reported in another paper).

While the hormone in mice and rats had given quite the same reactions as did the mares' serum gonadotrophin, its excretion in these animals and the guinea-pig tests showed that the two hormones were not identical. Other experiments made it reasonable to assume that its partial resemblance to mares' serum gonadotrophin was due to the fact that the hormone was a mixture of follicle-stimulating hormone (from the hypophysis of the patient) and chorionic gonadotrophin (from the tumor tissue). For comprehensive experiments on

*) Pyridin extract of human hypophyses.

**) Tannate from 24-hour urines, November 14—16.

the effect of mixtures of follicle-stimulating hormone and chorionic gonadotrophin had shown that a mixture of these hormones in a certain proportion gave exactly the same dose-response curves in infantile female rats as are obtained with mares' serum gonadotrophin. Thus a mixture of chorionic gonadotrophin and follicle-stimulating hormone in the proportion 1 I. U. chor. gon./ $\frac{1}{4}$ R. U. f.-s. horm. gave the same rat curves as were obtained with gonadotrophin from pregnant mares' serum (see Fig. 15, in which the reaction curves for the gonadotrophin from the patient are recorded too).

Also the histological changes in the ovaries showed complete conformity between 1) mares' serum gonadotrophin, 2) the mixture of chorionic gonadotrophin and follicle-stimulating gonadotrophin, and 3) the gonadotrophin from this patient; and it is possible with a fair degree of certainty to calculate that *the urine here concerned contained simultaneously about 300 I. U. of chorionic gonadotrophin and about 80 R. U. (= about 250 M. U.) of hypophysial follicle-stimulating hormone.*

Apparently the assumption of a simultaneous occurrence in the organism of chorionic gonadotrophin and hypophysial gonadotrophin is in conflict with certain experiences concerning the hormonal correlation. As is well known, the gonadotrophin production of the hypophysis is suppressed by chorionic gonadotrophin (cf. pregnancy, chorionepithelioma and cases of mixed epithelioma of the testis with high chorionic gonadotrophin production); but at the beginning of pregnancy the hormonal production of the hypophysis is normal, and a chorionic gonadotrophin concentration of the blood increases rapidly. In cases of testicular tumors the hypophysis often is in a strongly active secretory state, and the chorionic gonadotrophin production may be relatively slight. In some of our cases of testicular tumor, as mentioned, there was for a time an excretion of follicle-stimulating hormone suggestive of increased function of the hypophysis; later, however, the follicle-stimulating hormone was replaced by chorionic gonadotrophin which then had reached

a concentration high enough to inhibit the gonadotrophin production of the hypophysis. In the present case and in the analogous case from 1933¹¹⁾ the chorionic gonadotrophin

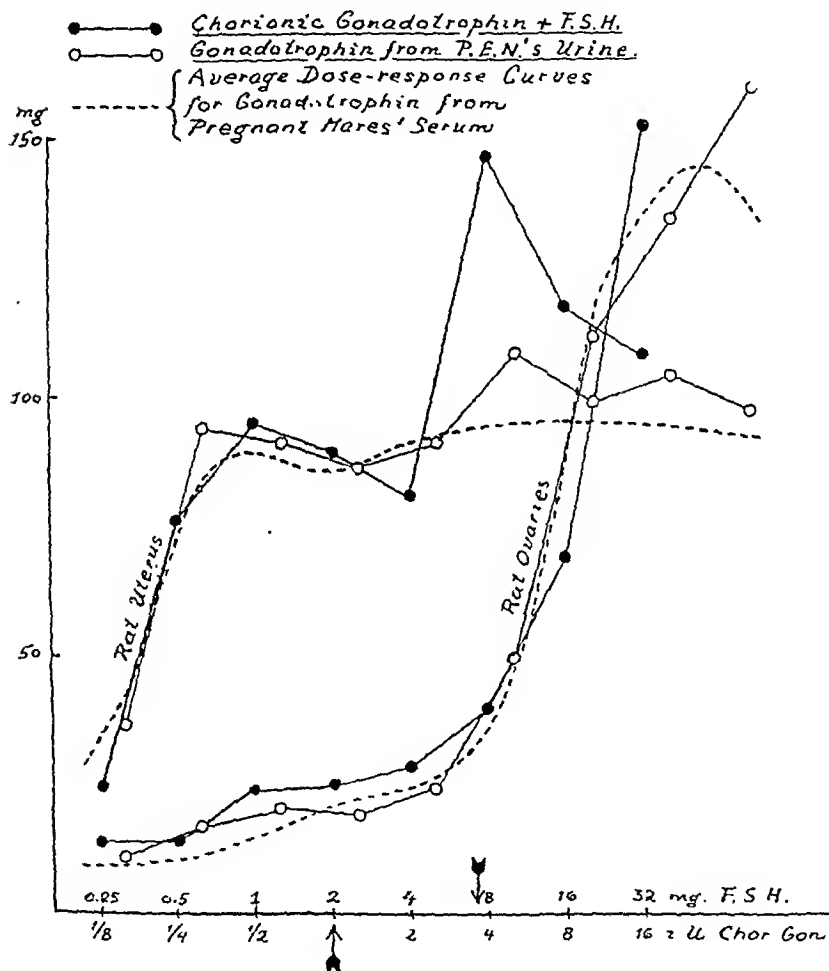


Fig. 15.

Dose-response curves for uterine and ovarian weights in infantile rats. The graph illustrates the concordance between 1) a mixture of chorionic gonadotrophin and follicle-stimulating gonadotrophin in the proportion: 1 I. U./ $\frac{1}{4}$ R. U., 2) the gonadotrophin from Case P. E. N., Nov. 1939, and 3) gonadotrophin from pregnant mares' serum.

evidently was not present in a sufficiently high concentration to suppress the gonadotrophin production of the hyperactive hypophysis.

Summary.

Of a material comprising more than 150 cases of malignant tumor of the testis that have been analysed hormonally, an account is given here of some cases in which particular studies were carried out on the nature of the gonadotrophin.

1) With normal infantile female rats for test animals, complete dose-response curves were worked out with 2 highly purified chorionic gonadotrophin preparations (Physex), with gonadotrophin from 3 cases of metastasizing mixed epithelioma of the testis, and with gonadotrophin from an extra-genital(?) chorionepithelioma in a man. These and other experiments with mice and rabbits showed complete concordance between chorionic gonadotrophin and the gonadotrophic hormone produced by these tumors.

2) In about 75 per cent of the cases of seminoma of the testis and small-cystic mixed tumor of the testis there was excreted with the urine a hormone which, according to the dose-response curves obtained on rats and the histological changes in the ovaries observed in infantile mice, was found to be identical with the hypophysial follicle-stimulating hormone present in castrate urine.

3) A detailed account is given of a case of malignant tumor of the testis, in which no increase could be demonstrated in the gonadotrophin excretion with the urine shortly after removal of the primary tumor; then, for a while, hypophysial follicle-stimulating hormone was excreted; and finally, at a later point of time, when large metastases had developed in the abdomen, the urine contained a mixture of hypophysial gonadotrophin (follicle-stimulating hormone) and chorionic gonadotrophin.

This mixture of gonadotrophins gave quite the same dose-response curves for ovarian and uterine weights in infantile

rats and mice as are obtained with gonadotrophin from pregnant mares' serum. But, when injected into an adult rabbit, 20 % of the gonadotrophin mixture was excreted with the urine in the first 24 hours after the injection. Of human hypophysial gonadotrophin and of chorionic gonadotrophin 12.5 % were excreted in rabbits, but of mares' serum gonadotrophin only 1.6 % — and even then only when injected in very large doses.

In experiments with mixtures of chorionic gonadotrophin and follicle-stimulating hormone in various proportions it was practicable to determine how much of each of these hormones was excreted with the urine in this case.

4) *In malignant tumors of the testis only two kinds of gonadotrophin are encountered: hypophysial follicle-stimulating hormone and chorionic gonadotrophin. In rare instances, these substances may be excreted successively in the same patient or, under quite particular conditions, simultaneously.*

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STUDIES ON THE EXCRETION OF ANDROGENIC SUBSTANCES AND GONADO- TROPHIN IN CASES OF MALIGNANT TUMORS OF THE TESTIS, ESPECIALLY SEMINOMA.

By Chr. Hamburger and Erik Godtfredsen.
(Received for publication January 10th, 1941).

In previous works of Bang, Hamburger and Jens Nielsen (1933—1941) it was shown that *seminoma of the testis* (and certain other malignant tumors of the testis) not infrequently is associated with excretion of follicle-stimulating gonadotrophin with the urine (see, for instance, Hamburger, 1941).⁴⁾ This hormone is to be regarded as being of hypophysial origin, as: 1) it has never been practicable to demonstrate this hormone in extracts of seminoma tissue (primary tumor or metastases), 2) the hypophysis of seminoma patients produces a strong gonadotrophin reaction when it is implanted in infantile mice, and 3) in its biological aspects the hormone is identical with the follicle-stimulating gonadotrophin found in castrate urine. Other testicular tumors — *mixed epithelioma* and *chorioepithelioma* — produce by themselves a gonadotrophin that is identical with the chorionic gonadotrophin (from pregnant women); in cases of this kind the hypophysis most often proves inactive when implanted in infantile mice.

In about 75 % of the cases of *seminoma of the testis* there

is a demonstrable increase in the amount of follicle-stimulating gonadotrophin in the urine (*i. e.*, from 50 to 400 M. U. per day), that is, the same incidence and quantity as *after castration* (Hamburger, 1933;²) E. Hart Hansen, 1941).⁵) The presence of the hormone is demonstrable before the removal of the primary tumor, and before the patient is given X-ray treatment; but the excretion of this hormone does not cease after hemicastration, and it is independent of the development of metastases. Excretion of this hormone is not a characteristic of testicular tumors alone; it has been demonstrated also in women with genital carcinoma, but in most of these cases it occurred during or after the climacteric period, when there is normally an increased excretion of follicle-stimulating gonadotrophin.

Besides the assay of gonadotrophin, in many of our cases of testicular tumor tests were made also for the excretion of *oestrogenic hormones* (Hamburger, 1938).³) In some cases of *mixed epithelioma of the testis*, with excretion of very large amounts of *chorionic gonadotrophin*, there was found to be also a considerable increase in the oestrin excretion (up to about 2000 M. U. per day). But, apart from these cases, the patients with tumors of the testis (including *seminoma*) showed no definite deviation from the conditions observed in normal men, the oestrin excretion varying from about 20 to 50 M. U. per day.

It has been rather difficult, however, to explain why the excretion of follicle-stimulating gonadotrophin is increased in tumor of the testis. The more obvious explanation would be: that the function of the testis in these patients was lowered to such an extent that in this respect they behaved like castrates. In the last couple of years, therefore, besides analysing the urine for gonadotrophin and oestrin we have determined also the amount of *androgenic substances* present in the urine in tumors of the testis, and in this way we have obtained some data that explain, at any rate in part, why seminoma patients excrete follicle-stimulating gonadotrophin.

The present paper will bring an account of these analyses and of various clinical examinations in several cases of *seminoma of the testis*.

Material.

From our entire material of hormonal-analysed testicular tumors the seminoma cases were picked out which all had been under treatment in the Radium Station, Copenhagen, and in which the diagnosis had been verified by Dr. Fridtjof Bang, pathologist to the Radium Station, and in which the quantitative hormonal analysis of the urine covered androgenic substances as well as gonadotrophin. These conditions were met in a total of 19 cases. In addition, the results are given for excretion of androgenic substances with the urine in 12 cases of mixed epithelioma of the testis and chorioepithelioma with excretion of chorionic gonadotrophin.

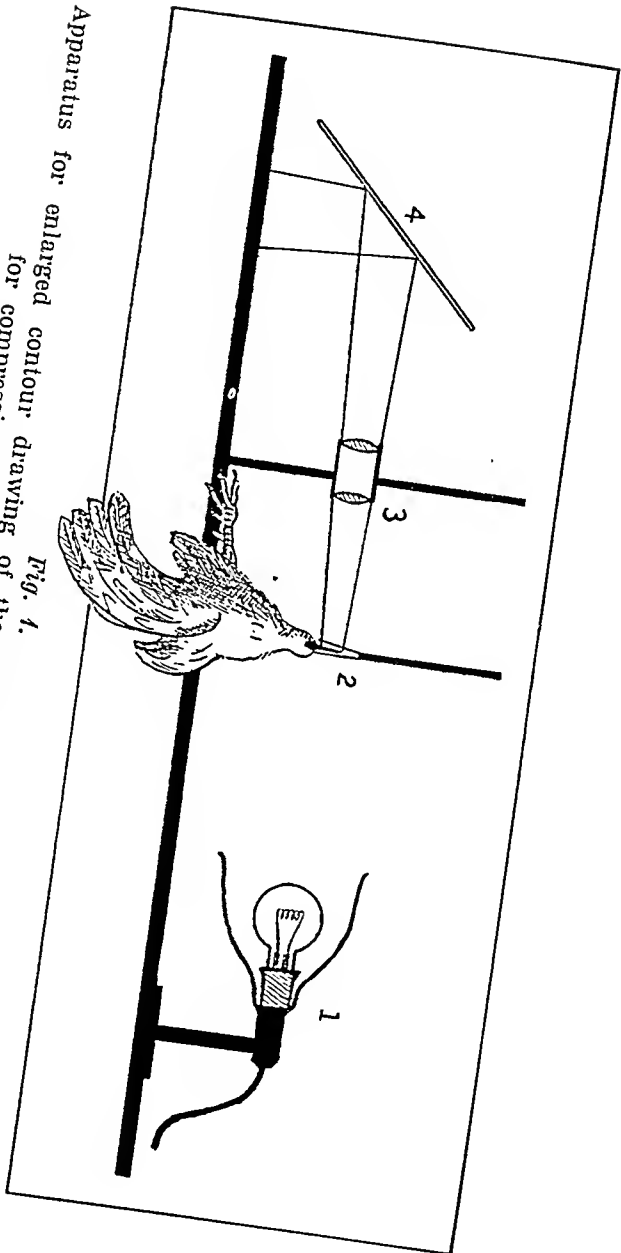
I. HORMONAL ANALYSES.

Technique.

Assay of gonadotrophin in urine was carried out after the hormone was precipitated by tannic acid, the precipitate washed with alcohol and acetone, and dissolved in water, with addition of sodium hydroxide to p_H 12. After neutralization the solution was injected into female mice, about 22 days old, distributed on 5 equal injections given in 48 hours; autopsy on the 5th day, with weighing and section of the ovaries. In the absence of an international standard unit, one mouse unit was established as the dose of hormone given an ovarian weight increase of about 50 %. The *qualitative* hormonal diagnosis is based on the histological changes in the ovaries (see, for instance, Hamburger, 1933).²⁾ Generally it was not considered essential to determine the exact excretion of follicle-stimulating gonadotrophin but merely to decide whether there was a definite increase in the excretion above the normal, *i. e.*, whether the excretion exceeded 50 M. U. per day.

Assay of gonadotrophin in tumor tissue was carried out after extraction of the minced tumor tissue with about 20 % aqueous *pyridin* under mechanical shaking; precipitation with 5 volumes of 96 % alcohol, washed with alcohol and acetone; drying, and

Apparatus for enlarged contour drawing of the capon's comb. 1. Projector. 2. Glass plates for compression of the comb. 3. Objective. 4. Mirror.



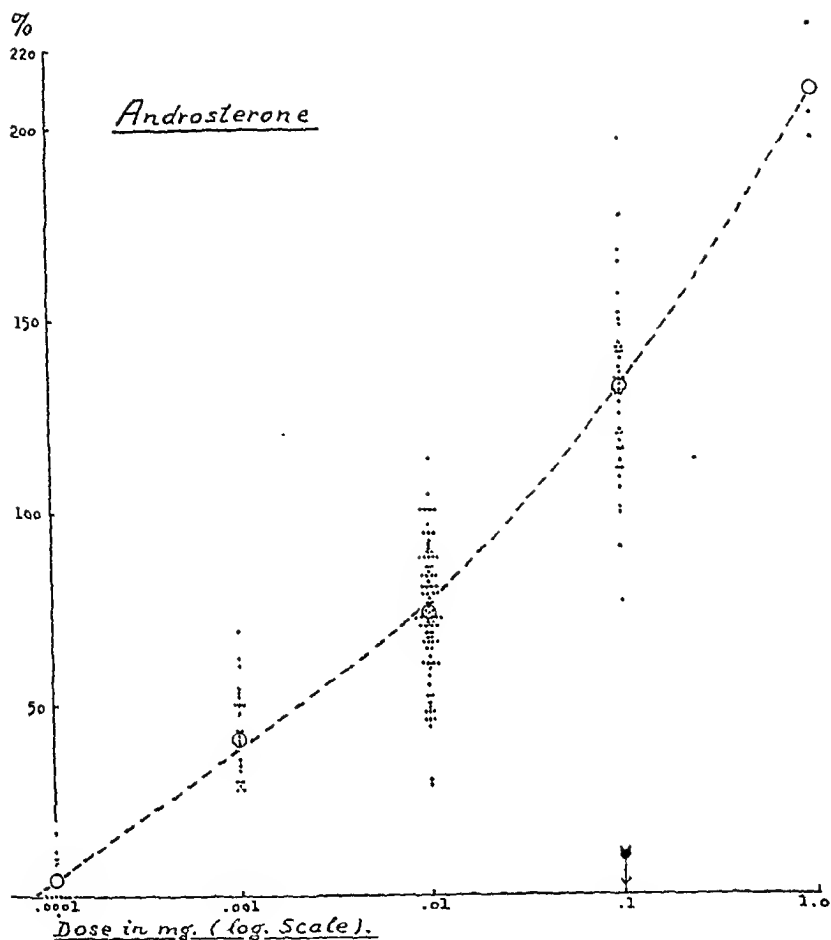


Fig. 2.

Dose-response curve for ANDROSTERONE, international standard preparation, dissolved in arachis oil. Local application to the capon's comb. single measurements (161 observations); O, average increase of the comb area (%).

solution in water with addition of sodium hydroxide to p_H 12. As a rule the biological assay of the neutralized tumor extracts was carried out on female rats, 26 to 28 days old.

Androgenic substances in the urine. One half of the 24-hour urine was extracted, after addition of 10 volume % of concentrated hydrochloric acid, 3 times 2 hours with benzene, on steam-boiler. After evaporation of the benzene the residue is dissolved in 10 cc.

Table 4.

Hormonal Analyses and Clinical Follow-up Data on 49 Hemicastrates (Seminoma of the Extirpated Testis).

Case No.	Age at operation	Metastases	Hormonal Analyses		Follow-up Examinations					
			Gonadotrophin *)	Androgen I. U. per day	Observation period after operation. (years)	General condition	Change in weight (kg.)	Blood cholesterol mg. %	Sexual life	
1	30	0	+	5, 6	6	Good	+ 1	212	Normal + Sp.	
2	31	0	0	30	7	Good	0		Normal + Sp. 3 children	
3	32	0	+	30	5	Good	0	167	Normal, 2 children	
4	32	0	+	10	12	Good	+ 20	204	Normal	
5	35	+ lumb. & supra-clavic. lymph gl.	+	3, 1	7/12	Fair	+ 4	154	Normal	
6	36	0	+	25, 6	5	Good	+ 5		Normal	
7	38	0	+	2, 20	4	Good	0	153	Normal + Sp.	
8	38	0	0	30	4/12	Good	+ 1		Normal	
9	38	+ lumbar lymph glands	+	4, 6	3/12	Good	0		Normal	

10	39	+ lumb. lymph gl.	+	8, 8	2	Good	+ 7	No data
					Died 4 years after operation. Autopsy: 0 metastases			
11	39	+ lumb. & pulmonary lymph glands	0	3	12	Good	+ 8	Normal
12	40	+ lumb. lymph gl.	0	4	4	Good	+ 9	Slight asthenia ejaculatiois
13	42	0	+	3, 2	4 1/2	Good	+ 4	Normal
14	46	0	+	11	2/12	Good	0	No data
15	48	0	+	1	2	Good	- 1	Normal
16	48	+ abdominal & mediastinal l. gl.	+	3, 1	10/12	Poor	- 12	No data
					Died 10 months after operation. Autopsy: + metastases			
17	50	0	+	30	8	Good	0	Normal
18	57	+ lumb. lymph gl.	+	4	4 1/2	Fair	0	No data
					Died 6 years after operation. with metastases			
19	59	0	+	4	4	Good	0	No data

*) + indicates that one or several examinations showed an excretion of ≥ 50 M. U. of follicle-stimulating gonadotrophin; 0 means that no analysis showed an excretion of 50 M. U. of gonadotrophin per day. Sp. = spermatozoa.

of arachis oil, and titrated by *direct application to the comb of castrated roosters* (white Leghorns), by daily application of 0.05 cc. of the oily extract (or a dilution of it) by means of a »tuberculin syringe« for 5 successive days. The comb area is measured at the first application and on the day after the last application by planimetric determination of a magnified shadow of the comb.

At first we employed the photographic method with weighing of the comb clipping. We then tried planimetric determination of this direct contact-copy of the comb, but this method was encumbered with too great errors of measuring. Finally we worked out a technique that gives a contour drawing of the comb, enlarged after a scala that allows planimetric measuring of the area. The arrangement is shown in Fig. 1. This method was employed in all the analyses mentioned here.

Each extract is examined in 2 doses (corresponding to $\frac{1}{80}$ and $\frac{1}{800}$ of the 24-hour urine) with 4—5 cocks per dose. The activity of the extract is determined by comparison with a dose-response curve for the *international standard preparation for androsterone* (Fig. 2).

The dose-response curve for dehydroandrosterone runs parallel to the curve for androsterone; but it takes about 5 times (by weight) as much of dehydroandrosterone as of androsterone to produce the same reaction. Likewise, the androgenic substances found in most of the male and female urines examined by us react in conformity with the same curve. In this way, the urines of *normal young and middleaged men* were found to contain on an average about 50 I. U. of androgenic substances per day, varying from about 20 to something over 100 I. U. per day — which results are quite in keeping with the findings reported by other investigators. In *male castrates*, E. Hart Hansen (1941)⁵⁾ found an excretion of androgenic substances corresponding to about 10 I. U. per day, varying from 5 to 18 I. U.; normal men showed on an average 40 I. U. per day (from 18 to 80 I. U. per day).

Analytical Results.

The results of the gonadotrophin and androgen determinations in the *seminoma patients* are recorded together with the clinical data in Table 1. As will be noticed, in 15 of the 19 cases of seminoma it was practicable by one or several analyses to demonstrate an increased excretion of *follicle-*

stimulating gonadotrophin. Only in 4 cases did the amount of *androgenic substances* in the urine fall within the normal limits (with 30 I. U. per day), and these cases were distributed over different age groups. In the remaining 15 cases the excretion was considerably lower (with 1 I. U. per day as minimal output). *In all 19 patients the average androgen excretion amounted to 10 I. U. per day — which is only one-fifth of the average excretion in normal men.* There was no correlation between the gonadotrophin excretion and the androgen output. Thus, for instance, in 7—8 years patient No. 11 was examined 7 times, and none of all the urine specimens showed any definite demonstrable increase in the gonadotrophin excretion, while the output of androgenic substances amounted only to 3 I. U. per day. In patient No. 12, too, there was no demonstrable increase in the gonadotrophin content of 5 specimens of urine analysed during the period of 4 years, while the output of androgenic substances was only 4 I. U. per day. Of the four patients with the greatest androgen excretion (30 I. U.) only 2 showed an increase in the gonadotrophin content of the urine in one or more analyses, while this was not observed in the other two. Thus, of all the 19 seminoma patients only two (Nos. 2 and 8) showed no deviations from the normal in any of the hormonal analyses. All the remaining seminoma patients behaved more or less like castrates as far as hormonal excretion is concerned.

From this analytical account it further is evident that the *gonadotrophin excretion is independent of the development of metastases*: in 10 out of 12 cases without clinically demonstrable metastases one or several analyses showed an increased excretion of follicle-stimulating gonadotrophin, while no increase in the gonadotrophin could be demonstrated in 2 of the 7 metastasizing cases. As to the *androgen excretion* it is to be emphasized that the 4 cases with about normal excretion were all free from clinical signs of metastases.

Only in very rare instances have we had an opportunity to carry out hormonal analyses on patients with seminoma

of the testis *prior to* the removal of the primary tumor. In two cases (including No. 1 in this account) there was an unquestionable increase in the excretion of follicle-stimulating gonadotrophin (≥ 100 M. U. per day) prior to the hemicastration. In one of the present cases (No. 5) the urine was examined 3 days before hemicastration and at that time no increase could be demonstrated in the gonadotrophin excretion, but the output of *androgenic substances* was very low (3 I. U. per day); 2 weeks after the operation the analysis showed 1 I. U. of androgen per day and still no increase in the gonadotrophin output; not until about 6 months after the hemicastration did the analysis show > 100 M. U. of follicle-stimulating gonadotrophin per day.

In two of the 19 seminoma cases a hormonal analysis was made of the tumor tissue too (the primary tumor in No. 5, and metastases in No. 16); no gonadotrophin could be demonstrated in the extracts — and this is in keeping with our previous investigations.

Besides in seminoma patients, the *androgen excretion* with the urine has been determined also in several cases of tumors of the testis (especially *metastases of mixed epithelioma*) associated with excretion of *chorionic gonadotrophin*. A comparison between the chorionic gonadotrophin output and the androgen excretion is given in Table 2. In one half of these 12 cases the androgen excretion fell within the normal limits; and in the remaining cases it was lower than normal. But, the *average androgen content of these urines was 21 I. U., that is twice as great as observed in the seminoma patients*. The most reasonable explanation of this finding is that the chorionic gonadotrophin has been able somehow to stimulate the androgen production of the remaining testis (cf. the results obtained with chorionic gonadotrophin therapy in hypogenitalism in male patients), but we cannot positively rule out the possibility that the tumor zones which produce chorionic gonadotrophin and, in some cases, oestrin too, might be able also to produce androgenic substances, or that the chorionic gonadotrophin might stimulate the suprarenals to

Table 2.

Excretion of Androgenic Substances in Chorionic Gonadotrophin-producing Tumors of the Testis.

Name	Age	Maximal excretion of chorionic gonadotrophin I. U. per day	Excretion of androgenic substances I. U. per day
O. C. H.*)	38	600.000	44, 32
P. U. D.	28	300.000	<1
E. H. S.	25	100.000	29, 25
L. G. S.	25	80.000	10, 20, 10, 2
K. H. J.	29	5.000	50, 15
N. C.	37	>1.500, <6.000	15
A. K.	31	3.000	65, 28
S. T.	28	3.000	28
P. T.	46	300	15
P. E. N.	25	300**)	5, 10
K. S. N.	38	≥ 150	2
Aa. M.	35	150	25, 16

*) Extragenital(?) chorioepithelioma.

**) + follicle-stimulating gonadotrophin.

increased secretion of steroid hormones. (The validity of the latter hypotheses may very likely be investigated further by studies on the excretion of androgenic substances in pregnant women).

II. CLINICAL STUDIES ON THE SEMINOMA PATIENTS.

As the outcome of the hormonal analyses was suggestive of a decreased testicular function in cases of seminoma of the testis it seemed appropriate to reexamine the 19 patients on whom hemicastration had been performed on account of seminoma — in order to see whether they would also present some clinical signs of genital hypofunction. A priori, however, we fully realized that even though there might be no demonstrable clinical sign of testicular hypofunction, such a finding would not exclude the possibility of an even considerable reduction in the functional capacity of the remain-

ing testis. As is evident from Sand's (1939)⁶⁾ investigations on 187 men who had been castrated after puberty, no absolutely characteristic »castrate type« may be set up. Sand's material, which in some respects has been investigated further by E. Hart Hansen (1941)⁵⁾, showed no changes in the growth of the individual, and only 15 % of the patients showed a gain in weight that had to be characterized as »castrate obesity«. The rate of standard metabolism was about 96 % of the normal. The general condition of the patients did not undergo any particular change; only during the first year after the castration were there frequent complaints of excessive sweating and hot flushes to the head. Loss of the hairs in the axilla was a frequent finding, also partial loss of the pubic hairs and atrophy of the prostate. Sexual desire subsided within a variable length of time, and gradually it subsided completely in about 90 % of the castrates examined. Further, Teilum (1940)⁷⁾ has shown that in male castrates there is a demonstrable increase of the cholesterol content of the blood. This increase makes its appearance after a latent period of about six months and continues up to 4—6 years after the castration, whereafter the value becomes constant. Only in rare instances is the blood cholesterol value particularly high, being usually about 250 mg. %, while in normal men the average was found to be 169 mg. %, with variations from 127 to 258 mg. %, and only in 15 % of the normal men did the value exceed 190 mg. %.

Method of Examination.

In keeping with the »follow-up system« of the Radium Station, all the patients have reported repeatedly for control examination after they had undergone the radiological treatment. In a majority of the cases the reexamination was not limited to a thorough clinical examination, including weight control, hæmoglobin determination, urine analysis and measuring of the blood pressure, but, in addition, they were submitted to roentgenographic examination of the lungs and

abdomen for estimation of the possible development of metastases. Furthermore the blood cholesterol concentration was determined in several of these cases, and microscopic examination for spermatozoa was made in some of them. For elucidation of their sexual life, the patients are questioned in particular about the duration of their matrimony, their number of children, the use of preventive measures and whether they have noticed any change in sexual desire or potency after the hemicastration and X-ray treatment. Further, of course, the patients are questioned about their general condition, energy, working capacity and possible variations of moods.

Data on the Patients.

The more important data on the 19 seminoma patients are given in Table 1, in which the patients are entered after age. Out of the 19 patients, aged from 30 to 59 years, 16 were alive and free from symptoms at the time when this material was worked up — in December 1940. Three patients had died respectively 10 months, 4 and 6 years after the hemicastration; two of these died with metastases in cachexia, while one died from some other cause; and in this case the autopsy revealed no metastases.

Of the 19 patients 7 had presented clinically or roentgenologically demonstrable *metastases*, mostly to the lumbar lymph glands, less frequently to the mediastinal and supraclavicular lymph glands and to the lungs. All the patients had been given *X-ray treatment*, from one to three series of irradiation applied to inguinal, abdominal and paravertebral fields. In the 7 cases with demonstrable metastases, these processes subsided on X-ray treatment. The *present observation period*, reckoned from the time of hemicastration, has varied from 2 months to 12 years; in 7 cases it has been 5 years or more, in 5 cases from 4 to 4½ years, in 2 cases 2 years, and in 5 cases from 2 to 10 months.

The *general condition* and working capacity were found

to be fully satisfactory in 17 cases, while 2 patients (Nos. 16 and 18) were feeling rather poorly; these two patients both died with metastases respectively 2 weeks and 1½ years after the last examination. Mentally, none of the patients has presented any change of depressive character.

Generally changes in the *weight* have been very moderate. No change was reported in 8 patients, while 9 showed a gain in weight, from 1 to 20 kg.; and only in three of these cases did the gain in weight amount to or exceed 10 % of the ideal weight (and the excess was very slight in 2 of these cases). Thus the gains in weight here reported are easily attributable to the abolition of the tumor or X-ray intoxication. A definite loss of weight was recorded only in 1 patient (No. 16), who was cachectic.

Only in one case was the *distribution of adipose tissue* localized to areas atypical of men: in No. 4 who gained 20 kg. in weight in the course of 10 years, the adipose tissue in the mammary region was very pronounced, but there was no true gynæcomasty. In no instance was there recorded any increased *sweat secretion* or typical *loss of hair* or decrease in the *growth of the beard*. In the 12 cases in which rectal exploration was performed at the reexamination, the prostate was found to be somewhat atrophic only in one.

Blood cholesterol determination was performed in duplicate (after Gørtz, 1934)¹⁾ in 9 cases — in every instance, more than six months after the hemicastration, and in 7 cases at least 4 years after. So, in judging of the values obtained it would seem safe to reckon that any eventual changes in the blood cholesterol concentration had made their appearance at the time of the analysis. *The obtained values have varied from 153 to 212 mg. %*; and only in two instances did the value exceed 200 mg. %, namely: 204 and 212 mg. %. These values *cannot be taken as definite evidence of real changes*, as the blood cholesterol level in any fairly large normal material shows a good many variations exceeding the limits given most frequently.

Information about the *sexual functions* is available in 14

cases, and in no instance is there recorded any change in the sexual desire or capacity. This information, however, is to be accepted only with all reservation possible, as certain conventional ideas naturally make the patients hesitate to admit any impairment or inferiority in these delicate respects. Concrete information about this point was obtainable in 4 cases: 2 of these patients (Nos. 2 and 3) had begotten respectively 3 and 2 children after the hemicastration (in both of these patients the androgen excretion was within the normal limits — with 30 I. U. per day — and only one of them showed once a slight increase in the gonadotrophin excretion). In two other cases (Nos. 1 and 7) with low excretion of androgenic substances and increased gonadotrophin excretion, besides in Pt. No. 2 the *semen* was *examined microscopically* (condom specimen); in all three patients the semen was found to contain spermatozoa of normal shape and in normal numbers, and no abnormal cells were observed.

Discussion.

The hormonal analyses carried out on the urines of 19 men in whom one testis had been removed on account of *seminoma* have confirmed our previous observation: that in about 75 % of such cases there is a demonstrable increase in the excretion of follicle-stimulating hypophysial gonadotrophin in the urine — in quite the same quantitative proportions and with the same frequency as in bilaterally castrated men. Like in a previous case, one of these patients showed an increased gonadotrophin excretion before the primary tumor was removed. The gonadotrophin excretion was further found to be independent of the presence of tumor tissue in the organism; it persisted after the removal of the primary tumor, and it did not increase even on the appearance of metastases from the seminoma; and the excretion was observed before the X-ray treatment was instituted.

So far it has not been practicable to explain by which mechanism the hypophysis was stimulated to increased gona-

dotrophin production in these cases. But on examination of the excretion of androgenic substances with the urine in these 19 seminoma patients (and a few other cases of seminoma outside the present material) the androgen content of the urine was found to be very low, the average output being 10 I. U. per day, while normal men excrete from 20 to a little over 100 I. U. per day, averaging about 50 I. U. Thus the androgen content of the urine of the seminoma patients was as low as that of the urine of male castrates.

After this it seems more reasonable to assume that the *gonadotrophin excretion* in patients with seminoma of the testis, past or present, is *secondary* in relation to a low androgen production — just like in castrates and in cases of hypogenitalism. On the other hand it is not possible with certainty to decide the cause of the presumably lowered production of androgenic substances. More likely it is due in part to the reduction in the total amount of testicular tissue of the organism either from the development of the tumor in one testis or from the hemicastration. (A few analyses of the hormonal excretion in men who had been *hemicastrated for reasons other than a tumor of the testis* and in whom there likewise was found a decrease in the androgen excretion and evidence of increased gonadotrophin production, lend support to this view). Further, it seems probable that a toxic effect from the tumor tissue in some cases — possibly in connection with the repeated applications of X-rays — may be a contributory factor in a reduction of the testicular hormone production, but not its sole cause.

The *clinical follow-up examination* of the seminoma patients revealed no definite evidence of any essential reduction in the functional capacity of the testis (determination of the blood cholesterol concentration, examination of the skin and hair, inquiry about the sexual capacity, examination of the semen, etc.). But this negative finding does not exclude the possibility of a lowered testicular function, as is evident from the fact that even bilateral castration after puberty in men does not give rise to the development of any characteristic type of castrates.

Summary.

Among 19 men who all had been hemicastrated on account of *seminoma of the testis* and who all had been given X-ray treatment in the Radium Station, Copenhagen, it was possible in 15 to demonstrate an *increased excretion of hypophysial follicle-stimulating gonadotrophin* with the urine. This excretion was independent of any development or absence of metastases. These patients also showed a *very low excretion of androgenic substances* (averaging 10 I. U. per day), i. e., only about $\frac{1}{2}$ of the daily output in normal men, at the low level of the daily output in male castrates.

Even though the *clinical follow-up examinations* of the seminoma patients failed to demonstrate any definite evidence of an essential reduction in the functional capacity of the testis, it is more reasonable to assume that the *increased production of hypophysial gonadotrophin in the seminoma patients is secondary in relation to a decreased production of androgenic substances*, which presumably is due in part to a reduction in the testicular hormone-producing tissue of the organism.

In 12 patients with chorionic gonadotrophin production (especially cases of mixed epithelioma of the testis and chorioepithelioma) there likewise was found to be a decrease in the excretion of androgenic substances even though the excretion here was twice as great as in the seminoma patients — something that is taken to be due to stimulation of the testicular androgen production by the chorionic gonadotrophin.

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A NEW PRINCIPLE FOR FORMALDEHYDE STERILIZATION OF SURGICAL INSTRUMENTS.

By *Gunnar Nordgren*.

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This article is a direct continuation of the author's earlier published work on sterilization by means of formaldehyde gas.*) In reference to the same I will restrict myself to a brief orientation of the subject. The practical inquiry is more closely dealt with towards the end of the article.

There are certain surgical instruments which can neither be sterilized by means of heat nor with the aid of chemical sterilization liquids which, as yet, have been available. For these there remains no other procedure than treatment with a bactericidal gas. As far as knowledge goes at present only one gas is unquestionable in this respect and that is formaldehyde.

Literature concerning formaldehyde's bactericidal effectivity under different conditions is remarkably comprehensive but full of conflicting reports. I began my investigation on the subject in 1937 due to the fact that formaldehyde sterilizations at Swedish hospitals, with comprehensive tests, had proved to be completely unreliable whichever method was used. None of the theories launched forth in literature as to the reason for the varying effectivity of formaldehyde under

*) *Nordgren*: Investigations on the sterilization efficacy of gaseous formaldehyde, *Acta path. et microbiol. scand., suppl. XL*, 1939.

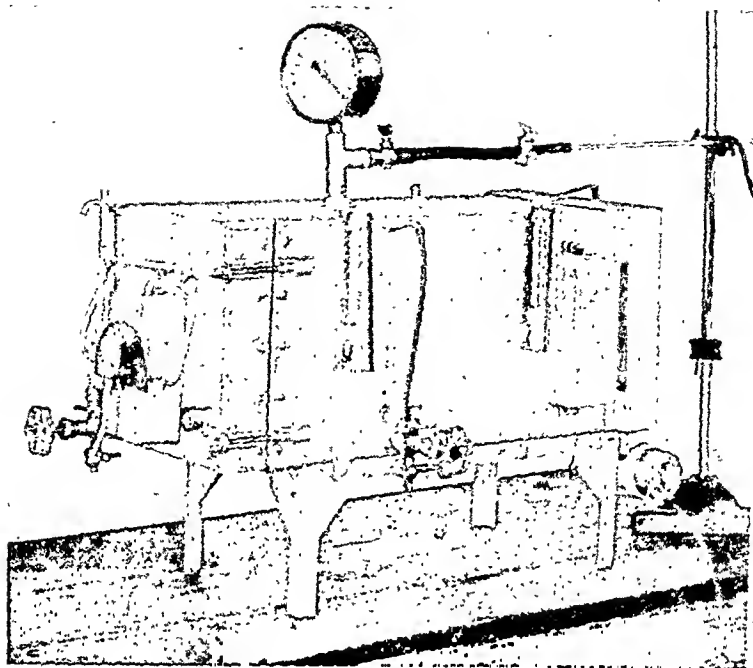


Fig. 1.

apparently similar conditions sufficed to explain the irregular, oftentimes very bad results of practical sterilization tests.

In the work above quoted I endeavoured to test experimentally the entire formaldehyde sterilization problem from the very beginning and I arrived at results which fully coincide with the physical gas laws and which apparently give complete explanation as to the irregularities and contradictions which have prevailed between the results of earlier investigators. In concluding the said work I put forth an idea of a new principle for formaldehyde sterilizations but with the omission of sufficient experimental demonstration for the proposed new principle's practical reliability.

However, since then, I have made both experimental and practical investigations of the principle and it is the results of the same which will now be put forward.

The instrument firm, *Stille* by name, in Stockholm, has

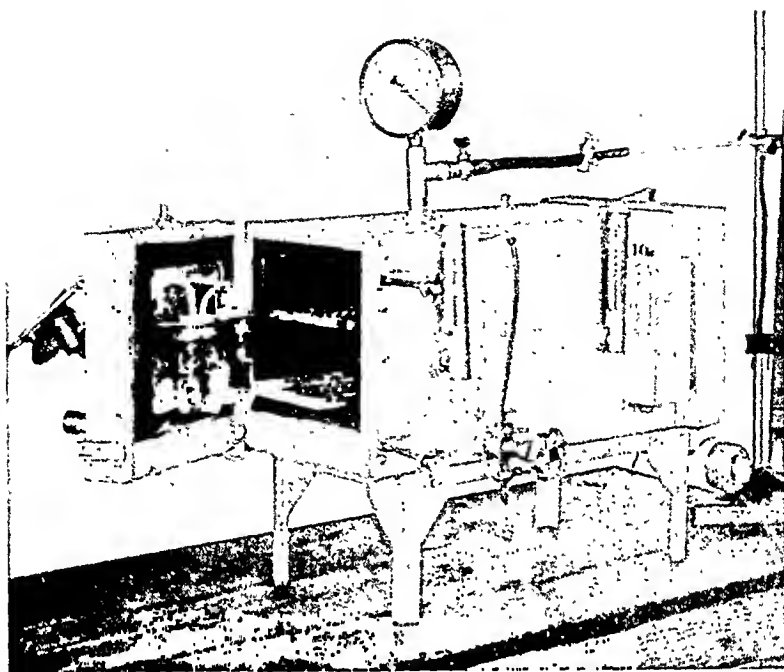


Fig. 2.

constructed a sterilizator for trial according to the author's instructions and said principle (Fig. 1—2). Most of the experiments mentioned in the following are executed by means of this apparatus and for this reason a brief description would seen in place here.

The sterilization apparatus. The sterilization chamber is 40 cm. long 15 cm. broad and 15 cm. high; it is surrounded by a water-mantle which can be heated electrically and which functions as a thermostat. The door of the chamber is also fitted with a water-mantle and has its own heating device. On the inside of the door are two containers for formalin through which air is allowed to stream when it is introduced after the evacuation. By turning on a tap on the outside of the door this same air, or ordinary air, can be made to enter the chamber. A pipe runs through the roof of the sterilizator and by this means the evacuation takes place. The pipe is at-

tached to a manometer on which the total gas pressure in the chamber can be read. In the chamber are two shelves of coarse-threaded metal wire capable of withdrawal, upon which articles for sterilization shall be placed.

EXPERIMENTS.

Two kinds of bakteriologic objects have been employed in the experiments, namely, objects with garden-soil and those infected with *b. subtilis* spores. Soil, according to general opinion, is the most difficult of all things to sterilize and by reason of this, sifted soil is commonly used as indicator of a sterilizator's effectivity. In sterilization tests with pure cultures *subtilis* spores can be regarded as representative of the most resistant groups of bacteria.

The comprehensive investigations which have shown that formaldehyde sterilization at hospitals in Sweden are completely unreliable have been executed with soil as indicator. For the sake of comparison, and to some great extent, I availed myself of precisely the same sterilization objects in experiments with the new method. Additionally have other tests been made with canals infected with soil or with *b. subtilis* as well as with infected catheters of various kinds.

A. Tests with the same apparatus which was employed in the earlier mentioned work.

Experiment No. 1. Table I. Temp. 60° C. Time 2 hours.

Objects: a) Woven catheter, 40 cm long, inner diameter 1.2 mm.						
b) Ureteral	»	, 74	»	»	»	1.1 »
c)	»	»	, 70	»	»	0.7 »

Following the infection, some centimeters of both terminals of the catheters were cut off and cultivated as controls for the bacteria percentage in their canals. After exposure to formaldehyde the catheters were cut in pieces, each some centimeters long, which, after being cleft lengthwise and treated with sulphite, were shaken with 50—60 graded agar, all then being poured into a Petri dish and allowed to set. Of the objects b and c were only alternate bits cultivated.

Results: all test bits were sterile.

		Table I.							
		a	b	c	d	e	f	g	h
Objects:									
Number of colonies									
on the control media:	500	126	300	67	107	1000	500	52	
	750	153	400	89	174	1400	600	101	
Media as in Exp. No. 1.									
at 0.9 mm									

Experiment No. 2, Table I. Same conditions as in Exp. No. 1.

Objects: d) Ureteral catheter, 75 cm long, inner diameter 0.9 mm
 e) " " " " " " " " 0.8 "

Alternate bits of the objects cultivated.

Results: all test bits were sterile.

Experiment No. 3, Table I. Same conditions as in Exp. No. 1.

Objects: f) Rubber catheter, 42 cm long, inner diameter 2.2 mm
 g) Woven " " " " " " " " 1.4 "

Alternate bits cultivated.

Results: all test bits were sterile.

Experiment No. 4, Table I. Same conditions as in Exp. No. 1.

Objects: h) Ureteral catheter 66 cm long, inner diameter 1.0 mm.

Alternate bits cultivated.

Results: all test bits sterile.

Experiment No. 5, Table II. Temp. 59° C. Time 2 hours.

Objects: a) Rubber catheter, 40 cm long, inner diameter 3.5 mm
 b) Woven " " " " " " " " 1.2 "
 c) Ureteral " " " " " " " " 0.7 "

Alternate bits cultivated.

Results: all test bits sterile.

Experiment No. 6, Table II. Same conditions as in Exp. No. 5.

Objects: d) Rubber catheter, 41 cm long, inner diameter 2.8 mm
 e) Ureteral " " " " " " " " 0.6 "

Alternate bits cultivated.

Results: all test bits sterile.

Objects:	Table II.				
	a	b	c	d	e
Number of colonies					
on the control media:	600	700	37	350	18
	1800	2000	61	600	29

Experiments Nos. 7—17. Objects: soil-packets.

Results: sterility was obtained at 60° C. in 2^h—1^h 20' (shorter times were not tested), at 55° in 1^h 30' and at 18—21° in 16 hours.

Owing to these good results the firm *Stille* constructed the sterilizator described in the introduction and I will now proceed to give an account of the experiments with this apparatus.

B. Tests with the sterilizator.

Firstly should be given those tests executed with soil as the object. As already mentioned, in Sweden, when sterilizators are controlled, small packets of sifted soil in filter-paper, wrapped in ordinary brown paper, are preferably used. Only in cases where the soil after treatment is absolutely sterile can a fully satisfactory effectivity be counted upon.

In the foregoing work I demonstrated that canals are specially difficult to sterilize with the aid of formaldehyde gas and in this respect a new tests method has now been submitted to experiment.

Glass tubes whose canals varied in diameter, namely, 1, 2, 3, 4 and 5 mm, were supplied with finely sifted soil so that the whole length of the canal contained soil, not however in any greater quantity transversely than that the greater part of the lumen remained free. Five pieces of such infected piping of the above calibres, all 30 cm long, were exposed together, and after the experiment the soil was shaken out of each into a Petri dish where was supplied firstly sulphite solution and afterwards liquid agar. The soil blended with the agar until the mixture showed a tendency to set. A couple of days were allowed to elapse in 37° thermostat and then was ascertained whether bacteria had developed or not.

Experiments Nos. 18—40. Objects: soil-packets and soil-infected canals. Temp. 60° C. Time 2 hours—50 minutes.

Results: all test objects sterile (115 soil-tests and 115 canal-tests). Control media overgrown.

Experiments Nos. 41—48. Table III. Same objects, same temperature. Times 45—30 minutes.

Results: all soil-packets sterile; canals: see Table III.

Experiments Nos. 49—59. Objects: soil-packets. Temp. 60° C. Time 20—5 minutes (5 Exp.s at 5 minutes).

Results: all the soil-packets, 55 in number, were sterile.

These results were beyond my most daring expectations: *sterilization of soil in packets in < 5 Min. at 60° C. must be considered a particularly good result.* On comparison with the experimental results in Chapter V given in my earlier work the above results are, however, found to be fully explicable.

Table III.

Exp. No.	Temp.	Time	Growth on the media				
			Canal calibre	1	2	3	4 5 mm
41	60°	45'		0	0	0	0
42	»	»		0	0	0	0
43	»	40'		0	0	0	0
44	»	»		+	0	0	0
45	»	35'		0	0	0	0
46	»	»		+	0	0	0
47	»	30'		+	+	0	0
48	»	»		+	+	0	0

Experiments Nos. 60—69. Table IV. Objects: soil-packets and soil-infected canals. Temp. 55° C. Time 1^h 30'—40':

Results: all the soil-packets sterile; canals: see Table IV.

Table IV.

Exp. No.	Temp.	Time	Growth on the media				
			Canal calibre	1	2	3	4 5 mm
60	55°	1 ^h 30'		0	0	0	0
61	»	1 ^h 15'		0	0	0	0
62	»	1 ^h		0	0	0	0
63	»	55'		0	0	0	0
64	»	»		+	0	0	0
65	»	50'		+	0	0	0
66	»	»		+	0	0	0
67	»	45'		+	+	0	0
68	»	»		+	+	+	0
69	»	40'		+	+	+	0

Experiments Nos. 70—86. Objects: soil-packets. Temp. 55—40° C.

Results: Sterility was obtained at 55° within 5 min. (15 tests), at 40° within 10 min. (15 tests).

Experiments Nos. 87—90. Objects: soil-packets and soil-infected canals. Temp. 17—21° C. Time 24, 20, 16 and 12 hours.

Results: All soil-packets sterile. The finer canals (cal. 1, 2 and 3 mm) were not sterile after 16 and 12 hours.

It now remained to execute tests with infected ureteral and urethral catheters as objects. The lumina of these were infected with suspensions of subtilis spores in a similar manner to that recorded in my earlier published work. In this case, however, I employed thicker spore-suspensions throughout.

Experiments Nos. 94—94. Table V. Temp. 60° C. Time 2 hours.

Objects:	a)	Rubber catheter, 42 cm long, inner diameter 2.8 mm				
	b)	Woven " , 37 " " , " " 2.0 "				
	c)	Ureteral " , 66 " " , " " 0.5 "				
	d)	" " , 71 " " , " " 0.6 "				
	e)	" " , 42 " " , " " 0.6 "				
	f)	" " , 74 " " , " " 0.7 "				

Results: all test bits sterile.

Table V.

Objects:	a	b	c	d	e	f
Number of colonies	1800	2000	250	95	43	50
on the control media:	2000	2500	270	130	68	70

Experiments Nos. 95—99. Table VI. Temp. 60° C. Time 1 hour.

Objects:	a)	Rubber catheter, 42 cm long, inner diameter 2.8 mm				
	b)	Woven " , 37 " " , " " 2.0 "				
	c)	Rubber piping, 40 " " , " " 2.5 "				
	d)	" " , " " " , " " 2.1 "				
	e)	" " , " " " , " " 1.6 "				
	f)	" " , " " " , " " 1.2 "				
	g)	" " , " " " , " " 1.6 "				
	h)	" " , " " " , " " 1.2 "				
	i)	Woven catheter, 37 " " , " " 1.6 "				
	j)	Ureteral " 54 " " , " " 1.9 "				

Results: all test bits sterile.

Table VI.

Objects:	a	b	c	d	e	f	g	h	i	j
Number of colonies										
on the control										
media:	450	210	700	650	350	170	140	72	100	45
	550	320	900	750	350	350	200	150	140	66

These results clearly testify to the capacity of the apparatus to sterilize catheters and even ureteral catheters of fine calibre in 1—2 hours at 60° C. It now became the question of endeavouring to fix the limits of the sterilization effectivity.

In this connection one can proceed with the idea that the sterilization capacity is in proportion to the time of exposure and to the temperature but in reversed proportion to the degree of infection i. e. percentage of bacteria. It can also be assumed that the sterilization becomes more difficult the longer the canal in comparison with its inner diameter. The following experiments were made.

Experiments Nos. 100—102. Table VII. Temp. 60° C.

Objects: a) Ureteral catheter, 74 cm long, inner diameter 0.4 mm
 b) " " , 67 " " , " " 0.7 "
 c) " " , 72 " " , " " 0.6 "

*Table VII.
Number of Colonies.*

Exp. No.	Object	Time	Controls	Pieces of the exposed catheters
100	a	1 ^h 30'	2500, 3000	0, 0, 0, 0, 0, 11, 92, 147, 52, 2, 0, 0, 0, 0,
101	b	2 ^h	1600, 1800	0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,
102	c	2 ^h	1100, 1500	0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,

From these experiments it is seen that ureteral catheters of fine calibre obviously become sterile in two hours at 60° C. in the apparatus even with a degree of infection corresponding to 1000—2000 highly resistant subtilis spores per 4—5 cm of their lumen, while absolute sterility seems not to be obtained under 1½ hour's exposure in the sterilizator at a higher degree of infection than 2000—2500 spores per 4—5 cm of the canal's lumen. Thus a good idea of the limit of the sterilizator's effectivity seems to have been gained. An exact fixation of this limit is of no practical importance as, in accordance with the above approximate determinations, it lies

sufficiently far from the conditions which could possibly arise in catheter sterilization in practice.

In practice one might safely assume that catheters or other canalized instruments which should be sterilized, as a rule, contain less than some hundred highly resistant spores per cm of their lumen. On the other hand it can not be excluded that canals, on one or other occasion can house a greater number of non-spore-forming bacteria. These are, however, considerably easier to kill than spores. Although this is well-known, the following complementary test was made.

Experiment No. 103. Temp. 60° C. Time 1 hour. Object: 72 cm long ureteral catheter, inner diameter 0.6 mm. This catheter was infected with a thick suspension of staph. aureus.

Result: Controls: >20,000 staph. per piece of the catheter. The whole of the exposed part was absolutely sterile.

On the basis of the above experimental results one should be justified in assuming that the sterilizator under practical conditions can be considered to have a fully reliable effectivity as regards all kinds of catheters and other canalized surgical instruments at a temperature of 60° C. and an operative time of 2 hours.

For canals of coarser calibre than approximately 1 mm., shorter than 30—40 cm., can a sterilization period of 1 hour at 60° generally be considered sufficient, and for simple surface sterilization 5 min. at 60° would suffice.

The practical importance of the experimental results.

The problem of formaldehyde sterilization, as already mentioned, has mainly consisted in the fact that, among other things, certain surgical instruments by reason of their material character or construction can not be sterilized by means of heat (dry or autoclave sterilization), nor have they been found adapted to sterilization through liquid chemical disinfectants.

The only harmless remaining method has been treatment with formaldehyde gas. Although formaldehyde sterilizers have proved to be insufficiently effective one has continued to use them because no other expedient existed. Thus, for instance, ureteral catheters have never been sufficiently sterilized and this has involved rather great risks. Such a catheter has been used in the one case in diagnosing kidney tubercle and in another for retrograde pyelography where virulent tubercle bacteria can have been ejected into a healthy kidney pelvis. For endoscopes in general no effective sterilization method has hitherto existed which is simultaneously harmless to the instrument, and consequently endoscopical investigations and operations have been combined with infectional risks.

Undoubtedly sterilization difficulties have exercised a considerable inhibitive influence upon technical development in the surgical field.

The sterilization problem exists even for the simplest surgical instruments. An ordinary knife, for instance, and still more, of course, a transplantation knife, is harmed both by dry and autoclave sterilization. For rubber gloves and catheters there has been no sterilization method either up to the present, which is both effective and harmless.

The formaldehyde sterilizer now described by the author should be capable of solving all the said sterilization questions. In the first place it can be used for sterilization of all kinds of compact objects (without inner canals or other hollows), when sterility can be obtained in 5 min. at 55—60° C. i. e. a temperature which all the material of surgical instruments can stand. If, in the future, some specific use should arise for a material specially sensitive to heat, this can be surface sterilized in 10 min. at 40° C., in the apparatus.

Short sterilization periods are of great practical value, of course.

As comparison can be mentioned the sterilization effectivity of the formaldehyde apparatus which has hitherto been considered the most effective at the Swedish hospitals. Such an apparatus has re-

cently been controlled at our laboratory. I personally tested it with the same objects i.e. the soil-packets and soil-infected canals, which were used in the previously given tests. It proved that for the sterilization of soil-packets at 60° C. a period of 1½ hours was required when formalin was used; but with paraformaldehyde which is mostly employed in practice sterilization was not obtained even after 3 hours. The soil-infected canals remained non-sterile in all tests.

The principle aim of my apparatus concerns canalized surgical instruments such as endoscopes, catheters, etc. The above experiments show, in this connection, that for canals of coarser calibre than 1 mm, shorter than 30—40 cm, a sterilization period of 1 hour at 60° C. can be considered reliable. This applies also to canals contaminated by dust from the air or the floor. All kinds of endoscopes hitherto used, even the sensitive prostataelectrotomes can be regarded as sterile in an hour in the apparatus without sustaining any damage. Similarly urethral catheters of every type can be effectively sterilized within one hour at 60°. For ureteral catheters the time must be extended to two hours owing to their greater length and finer canal calibre.

The above mentioned tests made with the hitherto most effective formaldehyde apparatus in Swedish hospitals proved its absolute ineffectivity to sterilize canals.

When the objects are removed from my apparatus after sterilization has taken place, evacuation follows and, afterwards, pure filtered air is let in. In this way there is no necessity for anyone to come in contact with greater quantities of formalin vapour, and the sterilization room is kept free from the same.

According to my experience little use is made of formalin in the apparatus. One can employ the ordinary technical formalin solution (35—40 %) and, as a rule, the containers need not be refilled until 75—100 sterilizations have been made. No condensation of paraformaldehyde in the containers was observed during the experiments. In tests with the other apparatus, however, the one now used in practice, there

occurred considerable condensation of paraformaldehyde in the containers, so that they had to be cleaned and refilled with fresh formalin already after 2—3 sterilizations.

As a standard control of the effectivity of formaldehyde sterilizers I would propose the following test:

3 small packets of soil and 3 glass tubes, 30 cm long with a 1 mm inner diameter containing a little dry, sifted soil, are placed in the apparatus. Following treatment in the usual manner the soil from both packets and tubes is cultivated on agar. Should colonies develop the sterilization has not been sufficiently effective.

Summary.

A formaldehyde sterilizer has been constructed on lines laid down by the author in an earlier published work (cf. footnote on p. 1).

During a large number of experiments this apparatus has proved to be exceedingly effective and in this respect exceeded my highest expectations.

At 55—60° C. — a temperature which is harmless to all surgical material — complete surface sterilization was obtained in less than 5 min.

At 40° C. surface sterilization was reliable after 10 min.

The time needed for surface sterilization with the hitherto most effective apparatus at Swedish hospitals is 1½ hours when formalin is used which, however, seldom occurs because it entails some disadvantages; paraformaldehyde, employed according to custom, requires more than 3 hours.

Compact objects such as sharp edged knives (transplantation knives), diagnosis endoscopes and other sensitive instruments of metal, rubber, ebony, glass (optical instruments) etc. can thus be sterilized in 5 min. in the apparatus, without sustaining damage.

Instruments with canals, for instance, operation cystoscopes become sterile in one hour at 60° C. and ureteral catheters in two hours at 60°. For such sensitive canalized instruments no sterilization method has hitherto existed.

A proposition concerning standard control is put forth.

STAPHYLOKOKKENSTUDIEN XVI.

WAS BEDEUTET DIE NEGATIV CHEMOTAKTISCHE SUBSTANZ FÜR DIE STAPHYLOKOKKENINFEKTION?

Von *J. Forssman*.

(Eingegangen bei der Redaktion am 5. Februar 1941).

Die starken Meinungsverschiedenheiten, die sich in den letzten Jahren hinsichtlich der Pathogenese der Staphylokokkeninfektionen geltend gemacht haben, brachten es mit sich, dass u. a. auch die negativ chemotaktische Substanz, welche die Staphylokokken angeblich besitzen, in den Vordergrund geschoben worden ist. Man hat sie für den entscheidenden Faktor der besagten Pathogenese und ebenso als Antigen für ausschlaggebend für die Immunität gegen die Staphylokokken gehalten.

Die in Rede stehende Substanz soll ja auf die mobilen Zellen, die Leukocyten, einwirken und sie dem Infektionsherde fernhalten, wodurch die Staphylokokken Zeit bekämen, sich zu vermehren und ihre Gifte zu erzeugen. Gelänge es dagegen den Leukocyten, an den Infektionsherd heranzukommen, so vernichteten sie (durch Phagocytose) die vorhandenen Kokken und erstickten dadurch die Infektion. Demgemäss behaupten die Vertreter der neg. chemotaktischen Substanz auch vorbehaltlos, »dass die Leukocyten den wirksamen keimtötenden Faktor gegen die Pyokokken ausmachen« (8).

Diese Ansicht über die Rolle der Leukocyten für die Bekämpfung der Staphylokokken fusst in der Hauptsache auf der Beobachtung, dass eine Injektion virulenter Staphylokok-

ken in mehrfach tödlicher Dosis intraperitoneal oder intrapleurale (nicht intravenös!) unwirksam (8) bleibt, wenn man ihr eine gewisse Menge Leukocyten beifügt. Hierzu ist zu bemerken: Da ein grosser Zusatz von Leukocyten zu einer Staphylokokkenaufschlammung *in vitro* nach allgemeiner Erfahrung eine sehr kräftig Phagocytose bewirkt, ist es nichts Aufsehererregendes — obwohl interessant — dass gleichfalls eine starke Phagocytose auftritt, wenn man dasselbe Gemisch in einen präformierten Hohlraum *in vivo* statt *in vitro* einführt, wobei die phagocytierenden Kokken vernichtet werden. (Wenn nun ihre Vernichtung ausschliesslich auf die Phagocytose zurückzuführen ist, was nicht als bewiesen gelten kann).

Nicht weniger interessant aber ist folgendes: Würde man das genannte Gemisch — Staphylokokken + Leukocyten — intravenös einspritzen, so würde sich der Leukocytenzusatz mit aller Wahrscheinlichkeit als wirkungslos erweisen und das Tier würde sterben, analog den Verhältnissen beim Milzbrand (8). Während hier nämlich eine intraperitoneale oder intrapleurale Einverleibung von Milzbranderreger und Leukocyten in passenden Proportionen die Tiere (Meerschweinchen und Kaninchen) fast unberührt lässt, ebenso wie die entsprechende Einspritzung von Staphylokokken und Leukocyten, bewirkte dagegen die *intravenöse* Injektion des Gemischs den Tod der Tiere ebenso schnell, wie die reine Bazillenemulsion, also ohne beigemischte Leukocyten. Hier sieht man also nicht die Spur eines Schutzes durch die Leukocyten. Somit zwei Versuche, die ganz entgegengesetzte Ergebnisse gezeigt haben, wo der Einverleibungsweg ausschlaggebend für den Erfolg ist und das günstige Ergebnis den besonderen Bedingungen zuzuschreiben sein dürfte, welche die präformierten Hohlräume Peritoneum und Pleura darbieten.

Man hat diese divergenten Ergebnisse damit erklären wollen, dass der Kontakt zwischen Leukocyten und Milzbranderreger (bzw. Staphylokokken) im Kreislauf zu wenig »intim« sei, so dass die Leukocyten keine Gelegenheit fänden, die Mikroorganismen zu vernichten. Dazu ist nun zu bemer-

ken, dass wir gar nichts darüber wissen, wie sich der besagte Kontakt in den Kapillaren gestaltet; er kann dort vielleicht recht eng sein, und die Phagocytose kann durch im Blute etwa vorhandene Stoffwechselprodukte verhindert bzw. gefördert werden. Zum Vergleich möchte ich, auf die Tatsache hinweisen, dass nach intravenöser Einspritzung von beispielsweise 150 Millionen virulenten Staphylokokken an Kaninchen die Zahl der Kokken im Blut in der Regel bereits nach 5 Minuten von ursprünglich 1.600.000 pr. cc. auf ein Hundertstel oder ein Tausendstel dieser Ziffer gesunken ist (2, 3). Und dies zum allergrössten Teil infolge der Tätigkeit von Milz und Leber. Also ein äusserst schnell ablaufender Vorgang im Kreislauf, wo der Kontakt zwischen Kokken im Blute und Retikuloendothel sich als ausreichend für die Aufnahme dieser Kokken erweise. Die Dinge liegen, mit einem Wort gesagt, nicht klar genug, um eine bestimmte Aussage hinsichtlich der Ursachen der in Rede stehenden Divergenz zu gestatten.

Gehen wir nun zur einfachen Staphylokokkeninfektion über, so wissen wir dagegen mit Bestimmtheit, dass die einfache tödliche Dosis je nach der Infektionsstelle erheblich verschieden sein kann; wo aber die Infektion auch erfolgt sein mag, die grosse Gefahr, die Lebensgefahr besteht bei Mensch wie Tier in dem Eindringen der Staphylokokken in die Blutbahn, wobei natürlich die Anzahl der eingedrungenen Staphylokokken für den Ausgang entscheidend ist. Der Hauptanteil der in den Kreislauf eingedrungenen Kokken wird dabei nachweislich von den Zellen des retikuloendothelialen Systems aufgenommen und wird — geeignete Dosierung vorausgesetzt — vernichtet, während die Leukocyten hierbei jedenfalls nur in äusserst geringem Umfang teilnehmen. Dies ist von mehreren Forschern, darunter auch von mir (3), nach intravenösen Injektionen von Staphylokokken an Kaninchen festgestellt worden; Ørskov (7) hat dasselbe auch bei Mäusen gesehen. Die Minderzahl von Kokken, die dann in Organe mit schwacher retikuloendothelialer Ausrüstung gelangt, hat dagegen gute Aussichten, sich zu entwickeln und neue Staphy-

lokokkenherde zu bewirken, von wo der Kreislauf erneut überschwemmt werden kann, die retikuloendothelialen Zellen erneut überfüllt werden können und der Tod evtl. eintreten kann.

Dementsprechend findet man nach intravenösen Staphylokokkeninjektionen beim Tode der Tiere *niemals* Eiterherde in Milz und äusserst selten in Leber — den retikuloendothelreichsten Organen — mögen die Tiere bald oder später nach den Injektionen verendet sein, während man dagegen Eiterherde *stets* in den Nieren und fast ebenso regelmässig im Herzmuskel sehen kann, wenn die Tiere wenigstens (2—) 3 Tage nach der Infektion fortgelebt haben. (Tritt der Tod innerhalb von 24 Stunden ein, so haben sich noch keine Eiterherde bilden können, obwohl die Nieren auch dann schon Staphylokokken enthalten). Und diese Eiterherde sind mit Leukocyten angefüllt, die also hinzugeströmt sind — der negativ chemotaktischen Substanz trotzend, wenn eine solche überhaupt existiert —, ohne jedoch die Infektion an Ort und Stelle hemmen zu können.

Ganz entschieden wird also die Phagocytose der in den Kreislauf gelangten Staphylokokken in erster Linie durch das Retikelendothel besorgt, was natürlich nicht verhindert, dass die Leukocyten sich an der Arbeit beteiligen, und dasselbe gilt von den retikuloendothelreichen Organen (Milz, Leber), doch übernehmen die Leukocyten den Kampf gegen die Staphylokokken nach ihren Kräften in steigendem Grade, wo der retikuloendotheliale Einschlag der Organe geringer ist.

Und im immunen Organismus verhält es sich nicht anders, nur dass die Phagoeytose in den betreffenden Zellen viel schneller vor sich geht (2).

Dass die Leukocyten keineswegs von entscheidender Bedeutung für die Immunität gegen die Staphylokokken sind, zeigen übrigens auch einige Versuche Flaums (1, S. 90). Drei Kaninehen (Nr. 123, 130 und 135), die er immunisiert hatte, bekamen eine tödliche Dosis Staphylokokken intravenös eingeimpft. Am Tage nach der Impfung wurde ihr Blut u. a. auf den Gehalt an weissen Blutkörperchen, speziell an Leuko-

cyten, untersucht. Es zeigte sich, dass die Tiere Nr. 123 und Nr. 135 6150 bzw. 5100 pro cmm hatten, also etwa wie ein normales Kaninchen, während Nr. 130 19.600 pro cmm hatte. Das letztere starb indessen von der Infektion am dritten Tage nach der Impfung, während die beiden andern überlebten. Ähnliches habe ich bei einigen eigenen Versuchen gesehen.

Die Behauptung, die »polymorphkernigen Leukocyten ausmachen das keimvernichtende Agens des Tierkörpers« gegen die Staphylokokken, ist somit irreführend; sie nehmen an dem Schutz gegen die Kokken teil; nichts weiter.

Bevor ich weitergehe, muss ich eine technische Frage abhandeln, die in dem vorliegenden Fall von der grössten Bedeutung ist. Wie wir sahen, spielt die *intrapleurale Einverleibung* der Staphylokokken bei den obengenannten Proben auf die Kraft der Leukocyten, die Entwicklung der Staphylokokken im Organismus zu hemmen, eine beherrschende Rolle, und dasselbe gilt auch bei den Versuchen, die Aufhebung der negativ chemotaktischen Substanz der Staphylokokken durch Staphylokokkenantiseren zu bestimmen (10). Angesichts dessen muss man sich die Frage stellen, welcher Wert solchen intrapleuralen Injektionen dann beigemessen werden kann. Pettersson ist selbst offenbar der Ansicht, sie lieferten äusserst regelmässige und zuverlässige Ergebnisse, entschieden bessere als die intravenösen Injektionen. Er schreibt: »es wurde absichtlich die intravenöse Einführung der Kokken vermieden, weil diese meiner Meinung nach recht variierende Resultate giebt« (10, S. 352). — Mit dieser Ansicht dürfte Pettersson doch ziemlich allein dastehen.

Die intravenöse Einverleibung der Staphylokokken, die Neisser in seiner grossen Monographie über diese Kokken (4) als die klassische bezeichnet, ist von den allermeisten Forschern auf diesem Gebiet (ich verzichte auch nur auf den Versuch, alle Namen aufzuzählen) als Virulenzprüfmethode angewandt worden, und zwar mit ausgezeichnetem Erfolg.

Pröseher (13) verwandte sie bei seinen Serumbestimmungen und fand sie »für eine exakte Bestimmung notwendig«. Er schreibt: »Wie ich mich in einer grossen Anzahl von Versuchen überzeugt habe, gibt die nach dieser Methode ausgeführte Wertbestimmung äusserst exakte Resultate.« Selbst habe ich mich dieses Einverleibungsweges bei Virulenzbestimmungen und Virulenzkontrollen bedient und mich im Laufe der Jahre an etwa 500 für diesen Zweck verwendeten Tieren von der Exaktheit der Methode überzeugen können.

Dass die intrapleurale Injektion exaktere Ergebnisse zeitigen sollte als die intravenöse, ist a priori absurd, *soweit nicht die Pleurahöhle operativ eröffnet und die Staphylokokkendosis direkt im Pleurasack deponiert wird*. Da Pettersson nichts darüber angibt, ob er sich dieses umständlichen Verfahrens bedient hat, muss man annehmen, dass er die intrapleurale Injektion ganz einfach mit einer gewöhnlichen Injektionsspritze, deren Spitze durch die Brustwandung gestochen wurde, ausgeführt hat. Zwar kann man annehmen, dass die Lunge einigermassen vor der Spritzen spitze zurückweicht, *besonders wenn diese stumpf ist*, doch muss man sich offensichtlich auf den *glücklichen Zufall* verlassen, damit die Spritzenöffnung ganz in die minimale Spalte mündet, die zwischen den beiden Pleurablättern verläuft. In vielen Fällen lässt es sich nicht vermeiden, dass die Spitze ganz oder teilweise entweder in der Intercostalmuskulatur bleibt oder jenseits der Pleura in die Lunge eindringt, mit dem Ergebnis, dass nur ein Teil der Flüssigkeit oder gar nichts in den Pleurasack gelangt; und damit sind die Resultate hochgradig verschieden.

Dass dies nicht nur theoretische Spekulationen sind, sondern dass es vielmehr den tatsächlichen Verhältnissen entspricht, dürfte, was die menschliche Pleura angeht, jedem Kliniker klar sein, der einige Erfahrung in Pleurapunktionen besitzt. Für die Kaninchenpleura wird dies in vorzüglicher Weise von Noetzel (6) beleuchtet. Er berichtet in seiner Arbeit über eine Menge intrapleurale Injektion von je einer bis dreizehn tödtlichen Dosen an diesen Tieren, wobei »jedesmal gleich-

zeitig gleich viele grosse, kräftige Tiere zur Hälfte mit, zur Hälfte ohne Pneumothorax infiziert wurden«.¹⁾ Durch Verwendung »einer stumpfen Kanüle (doch nicht stumpfer, als dass man die Haut durchstechen konnte)« glaubt er sich versichert zu haben, dass die Injektionen wirklich in der Pleura landeten, was sich indessen als ein offenkundiger Irrtum herausstellt. Bei der Sektion der mit Spritze und Kanüle pleurainjiziertem Tiere, 24 an der Zahl, zeigten 8 Abszesse an der Impfstelle, und von diesen hatten 2 ausserdem Abszesse an oder in der Lunge, 2 bloss Lungenabszesse und 1 Abszesse vor dem Perikard, während bemerkt wird, dass die *Pleura* bei 18 der 24 Tiere gesund, bei 11 von ihnen gar steril war; von den übrigen 6 Tieren hatten 5 nur einige Fibrinfasern und einen minimalen Erguss als Pleuraveränderung, und nur *ein einziges Tier* hatte ein grosses Empyem mit Reinkultur von Staphylokokken, was jedoch auch Noetzel selbst als das Ergebnis einer Fehlinjektion infolge einer »Impfverletzung« der Lunge betrachtet. Und dieses Ergebnis, während gleichzeitig *sämtliche* 26 parallelinjizierten Pneumothorax-Tiere, bei denen die Staphylokokkendosis *wirklich* in der Pleura deponiert worden war (wenn auch möglicherweise vor dem Zunähen etwas herausgesickert sein konnte), mehr oder weniger starke fibrinöse oder fibrinopurulente oder rein purulente Pleuritiden aufwiesen, die in 7 Fällen schnell zum Tode führten.

Die Verschiedenheit zwischen den mit Spritze und den nach Eröffnen des Brustkorbes infizierten Tieren erklärt allerdings Noetzel durch die ungleiche Technik für die beiden Gruppen ohne die Möglichkeit genug zu berücksichtigen, dass sie durch Fehlinjektionen zu verstehen war. Dass aber ein einfaches, momentanes Öffnen der Pleura eine solche tiefgehende Wirkung haben würde, ist erstens höchst unwahrscheinlich und dass es thatsächlich um Fehlinjektionen handelte, geht aus einem Vergleich mit den von Pettersson und

¹⁾ Die Pneumothorax-Tiere bekamen durchaus nur je 1 bis 3 tödtliche Dosen.

den gleich unten referierten, von mir vorgenommenen Pleura-injektionen offensichtlich hervor.

Hier ist demnach wahrscheinlich bei keinem einzigen der 24 mit Spritze und Kanüle intrapleural geimpften Tiere die volle Dosis in die Pleura gelangt; bei sämtlichen ist die Injektionsflüssigkeit entweder überhaupt nicht oder nur zu einem äusserst geringen Teil in der Pleura gelandet, sondern anderswo.

Um mir nun persönliche Erfahrung hinsichtlich der intrapleuralen Injektionsmethode zu erwerben, habe ich an insgesamt 16 Kaninchen bei verschiedenen Gelegenheiten diese Injektionen in ihrem Verhältnis zu und parallel mit der intravenösen (11 Kaninchen) geprüft; dabei habe ich vier verschiedene Staphylokokkenstämme benutzt.

Alle intravenös, mit je einer schnell tödlichen Dosis injizierten Tiere starben etwa nach der berechneten Zeit und wurden obduziert, wobei die für solche Fälle kennzeichnenden Obduktionsbefunde erhoben wurden. Die intrapleural, mit je einer bis fünf intravenös schnell tödlichen Dosen injizierten Tiere, die verendeten oder getötet wurden, wurden natürlich auch obduziert und besonders daraufhin untersucht, ob Anzeichen dafür vorlagen, dass die Injektionsflüssigkeit in der Muskulatur oder in der Lunge verblieben war. In zwei Fällen, wo die Tiere lebten, wo aber kein bzw. ein nur sehr geringer Gewichtsverlust den Verdacht erweckte, dass die Injektion an einer unrichtigen Stelle und nicht in der Pleura gelandet sei, wurden die Tiere nach 2—3 Tagen getötet, und bei der Obduktion zeigte es sich, dass die Injektion bei dem einem Tier in die Intercostalmuskulatur gegangen war, wo ein gelber, enorme Mengen von Staphylokokken enthaltender Knoten dies bezeugte, während die Pleura normal war, ohne Inhalt, und in dem zweiten Fall bestand zwar eine schwere Pleuritis, daneben aber ein grosses Infiltrat in der Lunge, welches Infiltrat enorme Mengen von Staphylokokken in Reinkultur enthielt. Es zeigte sich, dass ausser den beiden obengenannten Tieren noch weitere 2 der 16 intrapleural injizierten Tiere den Impfstoff in die Intercostalmuskulatur bzw. in die Lunge

(je ein Tier) bekommen hatten, und in einem weiteren Fall muss es unentschieden bleiben, ob nicht die Injektion zum Teil in die Lunge eingedrungen ist. Bei wenigstens einem Viertel aller so injizierten Tiere ist also die Injektionsflüssigkeit überhaupt nicht oder nur zu einem geringen Teil in der Pleura gelandet, sondern anderswo. *Und dies, ohne dass man eine Möglichkeit hat, bei der Injektion zu kontrollieren, wohin die Flüssigkeit gelangt*, und auch später, bei der Sektion, besteht nur eine sehr beschränkte Möglichkeit zu solcher Kontrolle.

Die übrigen 11 Tiere hatten serofibrinöse, fibrinopurulente oder purulente Pleuritis mit grossen Ergüssen, je nach dem Zeitraum zwischen der Impfung und dem Tode der Tiere.

Besteht also bei *intrapleuraler Injektion* keine Möglichkeit zu kontrollieren, wohin die Injektionsflüssigkeit wirklich kommt, so kann man hingegen ja bei der *intravenösen* Injektion z. B. in die Randvene des Ohres nach Entfernung des Haares und Waschen *ausnahmslos* deutlich sehen, wie die eindringende Injektionsflüssigkeit das Blut aus dem Gefäss zunächst verdrängt und dass nach der Injektion das Blut dann wieder zurückströmt, so dass man also bei dieser Form der Injektion *volle Gewissheit* erhält, dass der Impfstoff wirklich dem Blut zugeführt worden ist, also dahin kommt wofür er beabsichtigt war.

Angesichts dieser Umstände bei Titrationen und Kontrollen die intravenöse Methode zugunsten der intrapleuralen zurücktreten zu lassen, erscheint mir nicht angebracht, obwohl Pettersson aus seiner Erfahrung gute Erfolge mitzuteilen weiss. *Ich kann die intrapleurale Methode nicht anders als unsicher betrachten.*

Pettersson und vor ihm andere Forscher (4), z.B. der oben genannte Noetzel, haben, auf ihre Versuche gestützt, behauptet, die Kaninchen verträgen intrapleural beträchtlich höhere Dosen als intravenös. So gibt Pettersson die kleinste intravenös schnell tödliche Dosis mit $\frac{1}{2}$ Öse an, die entsprechende intrapleurale soll 12mal grösser sein, also 6 Ösen. In welchem Grade hier Fehlifikationen wie im Falle Noetzel mit-

gespielt haben, kann ich nicht sagen. Soviel glaube ich jedoch auf Grund meiner nicht ganz wenigen Versuche behaupten zu können, dass dieses Verhältnis bei verschiedenen Stämmen beträchtlich wechselt.

Nun zur Frage nach dem Vorhandensein der negativ chemotaktischen Substanz in den Staphylokokken und ihre etwaige Bedeutung.

Als Beweis ihrer Existenz führt man vor allem an, dass Tieren, denen eine innerhalb kurzer Zeit, z. B. 24 Stdn., tödliche Dosis Staphylokokken intrapleural einverleibt worden ist, ein leicht trübes, seröses Exsudat aufweisen, das durch hämolysierte Blutkörperchen leicht gerötet ist und einen sehr schwachen Fibringehalt hat. Die Armut an Zellen soll darauf beruhen, dass die negativ chemotaktische Substanz die Leukocyten daran hindert, herbeizuströmen.

Indessen ist diese Erklärung der Beschaffenheit des Exsudats keineswegs sicher oder auch nur wahrscheinlich. Untersucht man das besagte Exsudat ein wenig näher, so zeigt es sich, dass die leichte Trübung wenigstens zum Teil von Leukocyten herrührt, die wahrscheinlich hereingekommen sind und somit der vermeintlichen negativ chemotaktischen Substanz haben trotzen können. Die Beschaffenheit eines Ergusses beruht ja übrigens auf der Reizintensität und der Art der entzündlichen Gefässchädigung. Bei einer leichten Gefässchädigung sehen wir ein seröses Exsudat; siehe z. B. unsere rheumatischen oder tuberkulösen Pleuritiden mit in der Regel leichten Erscheinungen, die ja auch grossenteils ohne weiteres ausheilen. Nimmt die Heftigkeit des Reizes und damit der Gefässchaden zu, so verstärken sich parallel dazu sowohl der Fibringehalt als der Zellenreichtum des Exsudats.*) Ein serö-

*) Vergleiche hiermit die Verhältnisse bei den Pneumococcen. Diese Bakterien werden allgemein als wahrhaft negativ chemotaktische Mikroorganismen vorgezeigt. Und doch, siehe nur an die Pneumococcnpneumonien, wo ja von Anfang an und in immer steigenden

ses Exsudat, wie es oben kurz beschrieben wurde und wie man es nach Einführung grosser Staphylokokkenmengen in die Pleura sieht, braucht also nichts anderes zu bedeuten — und tut es sicherlich auch nicht —, als dass eine leichte pleurale Reizung als *Frühresultat* der Injektion bestanden hat. Wäre das Tier länger am Leben geblieben, so hätten Fibrin- und Leukoeytengehalt sicherlich zugenommen. Einen Eindruck davon gewinnt man, wenn man statt der genannten grossen Staphylokokkendosis mit einer kleineren Menge impft, so dass das Tier z. B. erst nach 3 Tagen stirbt. Dann sieht man nämlich ein Exsudat, das sowohl an Fibrin als an Zellen reich ist, und senkt man die Dosis noch mehr, so dass das Tier z. B. eine Woche am Leben bleibt, so finden wir bei der Sektion ein dünnflüssiges graugelbes Exsudat mit Massen von Leukocyten, die meisten nekrotisch, und die Pleura ist von einer ein bis zwei mm starken zottigen Fibrinmembran überzogen, die mit Exsudatzellen durchsetzt ist. Die Flüssigkeit und besonders die Oberflächenschichten enthalten grosse Mengen von Staphylokokken in Reinkultur. *Hier sehen wir also dieselbe Staphylokokkenaufschlammung, die in der grossen Dosis die seröse Pleuritis hervorrief, in kleinerer Dosis eine fibrinopurulente Pleuritis hervorrufen. Hier sind die Leukocyten nach Infektion mit der kleineren Dosis herbeigeströmt, während die grosse Dosis wegen des schnellen Verlaufs keine Zeit zur Hervorbringung ausreichender Mengen giftiger Stoffe für einen starken Reiz liess.*

Soweit ich sehe, stellt der erstgenannte Pleuraversuch keinen Beweis für das Vorhandensein einer negativ chemotaktischen Substanz in den Staphylokokken dar. Dass sich das Bild verändert, wenn man die Staphylokokkenaufschlammung mit einem Staphylokokkenantiserum versetzt, so dass Leukocyten hinzuströmen, ist eine vieldeutige Erscheinung, da das Serum teils selbst Leukocyten anzieht, teils der Einfluss der durch den Serumzusatz erhaltenen Stoffwechselprodukte nicht klar zu überblicken ist.

Mengen bis zur Krise die Leukocyten aus den Kapillaren in die Alveolen hinausströmen *ohne durch die negativ chemotaktische Wirkung der Pneumococcen verhindert zu werden.*

Als einen zweiten Beweis für das Vorhandensein einer negativ chemotaktischen Substanz in den Staphylokokken führt man an, dass man subcutane Staphylokokkenherde oft von einem klaren Ödem umgeben sieht. Da indessen im Zentrum solcher Herde, wo die Anhäufung von Staphylokokken am stärksten ist, auch Leukocyten in reicher Menge auftreten, so dürfte auch dieses Ödem als das Ergebnis einer leichten, vom Zentrum ausstrahlenden Reizung anzusprechen sein und nicht als das Ergebnis einer negativ chemotaktischen Substanz, die dann nämlich in erster Linie im Zentrum hätte vorhanden sein müssen, wo sich die Leukocyten schon angehäuft hatten. Wäre die Reizung stark und der Gefässschaden umfassend gewesen, so würde nämlich hier ebenso wie in dem intrapleurale Versuch mit grosser Staphylokokkennosis das Exsudat stark fibrinhaltig gewesen sein, selbst wenn die Leukocyten durch die vermeintliche negativ chemotaktische Substanz ferngehalten worden wären. Die seröse Beschaffenheit zeugt deshalb von einem schwachen Reiz und von nichts anderm.

Im Anschluss an die Besprechung dieser vermeintlichen Beweise für das Vorhandensein einer negativ chemotaktischen Substanz seien folgende Tatsachen unterstrichen:

1. Bei Phagocytenversuchen in vitro (Flaum, Pettersson) haben ebensowenig wie bei den Versuchen in vivo (Pleura- und Peritoneum-Versuche) irgendwelche Anzeichen für das Vorhandensein einer solchen Substanz festgestellt werden können. Flaum (1) hebt klar und bestimmt hervor, dass die Phagocytose pathogener und saprophytärer Staphylokokken äusserst kräftig ist und beide ungefähr *gleichermassen* betrifft.

2. Ein Zusatz von Immunserum steigert nach Flaum und Pettersson (12, S. 47) die wie gesagt schon ohne Serum äusserst intensive Phagocytose nicht merklich, eine Tatsache, die sehr schwer zu verstehen wäre, falls eine negativ chemotaktische Substanz vorhanden wäre, gegen die sich das Antiserum in ganz besonderem Grade richtete.

3. Nach einer von Pettersson angegebenen Methode hat er

gefunden, dass das vom Dänischen Seruminstitut hergestellte Standardserum gegen Staphylokokken die negativ chemotaktische Substanz von 10 mg Staphylokokken in einer Dosis von 0,000005 g neutralisierte. *Da 10 mg der verwendeten Züchtung 20 schnell tödlichen Dosen, intravenös eingespritzt, entsprechen, würde also 1 ccm des besagten Serums die negativ chemotaktische Substanz von 4 Millionen tödlichen Dosen neutralisieren* (12, S. 50). Zwar kann man nicht mit absoluter Übereinstimmung von gleichwirkenden Standardsera rechnen, wenn sie nicht aus demselben Aderlass und Mischung stammen, aber wenn sie wie hier im gleichen Institut und in gleicher Weise hergestellt worden sind und zudem die gleiche Aichung hatten, muss doch die Übereinstimmung sehr gross sein. Es ist daher als sehr merkwürdig zu bezeichnen, dass wie Flaum (11, S. 109) gezeigt hat *die Injektion von 5 ccm, ja sogar 7,5 ccm solchen Standardserum intravenös ein Kaninchen nicht einmal gegen eine einzige intravenöse schnell tödliche Dosis zu schützen vermochte*. Das wäre undenkbar, wenn die sog. negativ chemotaktische Substanz der entscheidende Faktor für die Pathogenese und als Antigen auch der ausschlaggebende Faktor für die Immunität gegen die Staphylokokkeninfektion wäre.

4) Der Umstand, dass nach intrapleurale Injektionen von grossen Dosen Staphylokokken in Mischung mit Leukocyten die so injizierten Tiere nicht einmal erkrankten und dass dies dadurch erklärt wird, dass »die Staphylokokken schnell phagocytiert werden« (Pettersson, 11) spricht ja nicht für sondern gerade gegen die Annahme einer negativ chemotaktischer Substanz.

Es soll noch folgendes hervorgehoben werden. Allgemein anerkannt ist, dass die minimal tödliche Dosis von Staphylokokken beträchtlich höher ist, wenn die Staphylokokken intrapleural oder intraperitoneal bei Kaninchen eingeführt wird als man sie intravenös injiziert. Oder umgekehrt die Tiere sind viel empfindlicher für intravenöse als für intrapleurale oder intraperitoneale Injektionen.

Dies muss doch den Anhängern von der Meinung, dass

die Staphylokokken negativ chemotaktisch sind, sehr auffallend oder sogar merkwürdig vorkommen. Wie anfangs schon gesagt, meinen ja diese Forscher, dass »die Leukocyten das keimtötende Agens gegen die pyogenen Staphylokokken ausmachen«. Da nun diese Zellen durch die negativ chemotaktische Substanz der Staphylokokken ferngehalten werden und wir in Pleura oder Peritoneum keine andere Faktoren kennen, die die Staphylokokken zu bezwingen vermögen, würden die Staphylokokken in diesen Höhlen die Gelegenheit haben sich *unbeschränkt zu vermehren*, während dieselbe Dosis, intravenös injiziert im grössten Umfang vom Retikuloendothel schnell phagocytiert und vernichtet wird. Die kleine übriggebliebene Rest muss sich dann erheblich vermehren, um die nötige Zahl zu erreichen, die das Tier tötet. Hierdurch wird ja den intrapleural oder intraperitoneal eingeführten Kokken ein grosser Vorsprung vor den intravenös injizierten gegeben und vom diesem Gesichtspunkte sollte die minimal tödtliche Dosis in jenen Fällen *erheblich kleiner* und nicht, wie die tatsächlich ist, *grösser* sein.

Gehen wir zur Betrachtung von den entsprechenden Verhältnissen bei einer *wahrhaft* negativ chemotaktischen Bakterie — dem Pneumokokkus — über, so sehen wir auch, dass es da sich so verhält. Es ist nämlich schon seit langem hier bekannt, dass die intraperitoneal tötliche Dosis ungeheuer viel geringer ist als die intravenöse. Ørskov (14) giebt neulich ein vorzügliches Beispiel davon. Von demselben Pneumokokkustamm injizierte er bei derselben Gelegenheit 10 Mäuse intravenös mit je 50000 Pneumokokken und anderen 10 Mäuse intraperitoneal mit je 5 Pneumokokken. Von jenen überlebten 8 und nur 2 starben, von diese dagegen starben alle 10.

Dieser Vergleich spricht gegen die Auffassung, dass die Staphylokokken unter den negativ chemotaktische Microorganismen zu rechnen sind.

Nach dem oben Gesagten kann ich nur feststellen, dass keine Beweise für das Vorhandensein einer solchen Substanz in den Staphylokokken erbracht worden sind und dass viel sogar gegen die Existenz der fraglichen Substanz spricht.

In der Diskussion um die Frage des vermeintlichen Vorkommens der negativ chemotaktischen Substanz bei den Staphylokokken weist man immer wieder auf die Verhältnisse bei den Pneumokokken und beim Anthrax als Parallelfälle hin. Wie die meisten Vergleiche, so hinken auch diese, und zwar in solchem Masse, dass man sich versucht fühlt, sie eher als Gegensätze aufzustellen, was Virulenz und Immunität angeht. So sind die Staphylokokken niedrigvirulent. Um ein Kaninchen innerhalb von 24 Stunden durch *intravenöse* Injektion zu töten, sind im besten Falle und bei Verwendung der virulentesten Staphylokokkenstämme 70—100 Millionen Staphylokokken erforderlich, während einige wenige hochvirulente Pneumococci oder Anthraxerreger ein Kaninchen schnell zum Tode führen; kommt dazu die grosse Verschiedenheit bei den intrapleurale oder intraperitonealen Injektionen im Vergleich zu den intravenösen, wovon eben gesprochen ist. Und was die Immunität angeht, so kann man bei den Staphylokokken mit *intravenöser* Injektion kaum über die aktive Immunität von 20 Dosen hinauskommen, während die aktive Pneumokokkenimmunität bei Mäusen sicher die 100 000 000fache tödliche Dosis beträgt. Auch bei der passiven Immunität gegen diese beiden Microorganismen sehen wir ebensogrosse Verschiedenheiten. Diese Unterschiede sowohl in der Virulenz als in der Immunität sprechen für sich und müssen m. E. vor dem Ziehen von Parallelen warnen. Angesichts solcher Tatsachen sind die Übereinstimmungen sicher nur oberflächlicher und keineswegs wesentlicher Natur.

Zusammenfassung.

1. Die Behauptung, die Leukocyten seien der Schutz des tierischen Organismus gegen die Staphylokokkeninfektion, ist irreführend. Die wichtigsten Zellen im Kampfe gegen die Staphylokokken sind die Zellen des retikuloendothelialen Systems; die Leukocyten sind nur wertvolle Hilfstruppen.

2. Dass die Staphylokokken eine negativ chemotaktische Substanz ausscheiden, ist unbewiesen, und sowohl Phagocy-

toseversuche in vitro als Pleuraversuche mit einem Gemisch von Staphylokokken und Leukocyten lassen die Existenz einer solchen Substanz als unglaublich erscheinen.

3. Im gleichen Sinne spricht auch der mangelnde Einfluss von Immunseren auf den Grad der Phagocytose sowie die mangelnde Fähigkeit sogenannter stark anti-negativ-chemotaktischer Immunseren, auch in grossen Dosen die Staphylokokkeninfektion nach intravenösen Injektionen verhältnismässig kleiner Kokkendosen zu beeinflussen.

4. Die intrapleurale Injektionsmethode, die bei den Untersuchungen über das eventuelle Vorkommen von einer negativ chemotaktischen Substanz der Staphylokokken grosse Verwendung gefunden hat, ist eine unsichere Methode.

Die Untersuchung ist mit ökonomischer Hilfe der Stiftung »Therese och Johan Anderssons Minne« ausgeführt.

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FROM THE DANISH ANTI CANCER LEAGUE'S CANCER
RESEARCH LABORATORY.

(DIRECTOR: J. ENGELBRETH-HOLM, M. D.)

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THE EFFECTS
OF 9:10-DIMETHYL-1:2-BENZANTHRACENE
ON TRANSPLANTED TUMOURS.

By S. Stamer.

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In 1935 Haddow published some experiments for the purpose of showing that 1:2:5:6-dibenzanthracene, 3:4-benzpyrene and other carcinogenous hydrocarbons had an inhibitory effect on the growth of Jensen's rat sarcoma and the Walker carcinoma and also on a sarcoma induced by 1:2:5:6-dibenzanthracene. He was unsuccessful in stopping the growth when he used related hydrocarbons that are not carcinogenous. Later, Haddow and Robinson (1937) described how many carcinogenous hydrocarbons had this inhibitory effect on the growth of tumours, one that was also shared by weak carcinogenous hydrocarbons. They also found that rats treated with the carcinogenous hydrocarbons grew much more slowly than the control animals.

In 1936 Morelli confirmed that 1:2:5:6-dibenzanthracene and 3:4-benzpyrene had a marked inhibitory effect on transplanted tumours. Inhibition was greatest when the treatment was given simultaneously with transplantation. These results were in turn confirmed by Lees (1937), who treated rats, on which Jensen's rat sarcoma had been transplanted, with intra-abdominal injections of 1:2:5:6-dibenzanthracene. Pollia (1938) also found that this product had an inhibitory effect on transplanted rat sarcoma. Alapy (1938) reported on prophylaxis with very small doses of benzpyrene, dibenzanthracene and methylcolanthrene (15 to 3). He obtained the best results when the hydrocarbons were injected thirty to forty days prior to transplantation. Inhibition was only slight in mice, however, and scarcely measurable in rats. Pybus and Miller (1936) tested the effect of 1:2:5:6-dibenzanthracene on spontaneous mammary tumours in mice and found that there was a temporary in-

hibition of the growth of the tumours and in some cases even a temporary recession; in all cases, however, the tumours commenced to grow again after four or five weeks.

Bauer (1937) treated malignant skin tumours in man with 3:4-benzpyrene. He employed a 0.5 % ether solution of the hydrocarbon, injected into the growths in doses of 0.5 to 1 c.c.; he also sprayed the surface of the growths with this solution. Seven out of 23 cases are stated to have been free from relapse for more than two years. Peacock and Beck (1938) report on inhibition, in fact on occasional total regression, of transplanted mouse carcinomata after intravenous injections of 3:4-benzpyrene.

In contrast to all other workers, Appel, Strauss, Kolischer and Nacheles (1938) found that subcutaneous injection of 1:2:5:6-dibenzanthracene stimulated the growth of the Brown Pearee rabbit carcinoma, and that metastases came earlier, and indeed more frequently in organs which seldom are affected by metastasis otherwise. In 1939 Haddow and Robinson published a long series of experiments in which they had tested the effect of 34 carcinogenic and 34 non-carcinogenic hydrocarbons on the Walker carcinoma, Crocker sarcoma and spontaneous mammary tumours in mice. In about 87 % there was inhibition of the tumour growth when treated with carcinogenic hydrocarbons, and in only 13 % was there no inhibition.

A more interesting feature was, however, that in about 25 % there was inhibition when treated with non-carcinogenic hydrocarbons. Accordingly, the inhibitory effect does not seem to be associated solely with the carcinogenic property of the hydrocarbons.

It should be observed that in all these experiments the carcinogenic hydrocarbons were applied by injection subcutaneously or intraabdominally; intravenous injection was employed only in the experiments of Peacock and Beck.

A comprehensive survey of the inhibitory effect of the carcinogenic hydrocarbons on malignant tumours was published by Stamer in 1940.

Own Experiments.

The writer's experiments were all made with 9:10-dimethyl-1:2-benzanthracene. This hydrocarbon was synthesized by Newmann (1938) and by Bachmann and Chermersda (1938) as well as by Mikhailow and Tschernova (1938). Its carcinogenicity was tested by Bachmann, Kennaway and Kennaway (1938) and by Shear (1938) by subcutaneous injection into mice of the product dissolved in oil.

The reason why 9 : 10-dimethyl-1 : 2-benzanthracene was chosen from among the many known carcinogenous hydrocarbons was that this had the strongest carcinogenous activity of them all. According to the experiments referred to above, carcinogenous hydrocarbons have a definitely inhibitory effect on malignant tumours, and therefore it was natural to examine how the strongest carcinogenous hydrocarbon would act on growth, as a priori it was to be expected that the greater the carcinogenous activity, the greater must its inhibitory effect be.

For these experiments the animals used were mice of Little's dilute brown strain (Dlb.), with transplanted spontaneous mammary carcinomata, and mice of a Danish strain known by the name of »Street«, with transplanted Crocker sarcoma 180. Both forms of tumour have 100 % of takes in these strains. »Spontaneous« regression is observed only in the event of the transplanted tumour being infected.

Mammary Carcinoma in Strain Dlb.

In an experimental test a spontaneous mammary carcinoma from a Dlb. mouse was transplanted to 12 Dlb. mice. The tumour established itself in all the animals, and, 27 days later, when the tumours were about 2 c. c. in size, the animals received 1 mg. 9 : 10-dimethyl-1 : 2-benzanthracene in 0.1 c.c. Ol. olivae injected intra-abdominally, whereas the control animals received 0.1 c.c. Ol. olivae. Sixteen days later the animals were killed, as the tumours in the control animals threatened to ulcerate. At this time the experimental animals were in poor condition, the fur tousled, and they were visibly smaller than the control animals. The tumours in the experimental mice were stationary or slightly diminished compared with those of the control mice. The latter were in good condition, the fur smooth, and they had all put on weight. The tumours in these animals had all grown steadily.

The size of the tumours and the weight of the animals are shown in Table 1 and Fig. 1.

Table 1.

	Average Body weight	Average Tumour size
Experimental animals.		
Injection day	22 gr.	2.87 c. c.
16 days later	17 gr.	2.21 c. c.
(loss 22.7 %).		
Control animals.		
Injection day	20.2 gr.	1.69 c. c.
16 days later	21.2 gr.	4.59 c. c.

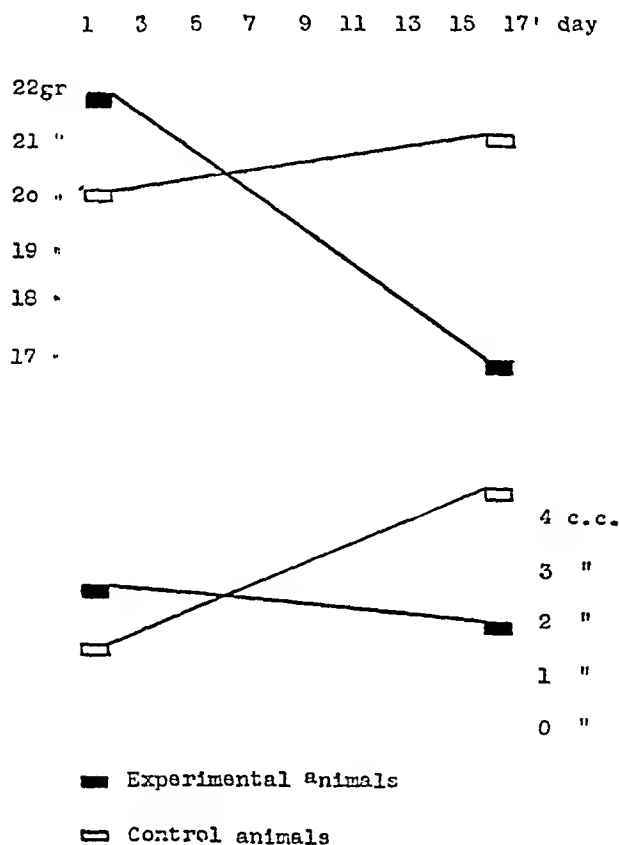


Fig. 4.

Table 2.

	Experimental Animals		Controls	
	Weight gr.	Measurement c. c.	Weight gr.	Measurement c. c.
Injection day	15.1	0.5	14.8	0.5
3rd day	14.2	1.1	13.5	0.6
5th day	14.6	1.0	15.2	0.9
7th day	13.7	1.5	13.9	1.3
9th day	14.9	2.3	15.3	1.7
10th day	14.5	2.8	15.2	1.9
12th day	15.1	3.6	13.9	2.6
14th day	15.1	4.2	14.8	4.0
16th day	15.0	5.6	16.0	3.9
17th day	16.1	5.7	16.7	5.6
19th day	16.8	4.0	17.8	4.9
21st day	15.6	6.1	16.4	6.0

The measurements of the tumours expressed in cubic centimetres were obtained by measuring them in all three dimensions. Naturally the result is somewhat inexact, but it is the only one available.

Apart from the tumours the autopsy revealed nothing abnormal. There was no metastasis in any animal. Histological examination of lungs and kidneys showed nothing abnormal, whereas the liver of the experimental animals presented slight parenchymatous degeneration.

Thus in this experiment the growth of the tumours was distinctly inhibited, but it also appeared that the dose of 9 : 10-dimethyl-1 : 2-benzanthracene was too large, it having had a toxic effect on the animals. As will be seen from Fig. 1, they lost 22.7 % in weight on an average, and they were in poor condition. The histological examination also showed that the injection had injured the liver.

In the next experiment the attempt was therefore made to administer such a small dose that its effect would not be toxic. Twenty Dlb. mice were planted with a spontaneous mammary carcinoma from another Dlb. mouse. Seventeen days after transplantation the mice were given 0.1 c.c. olive

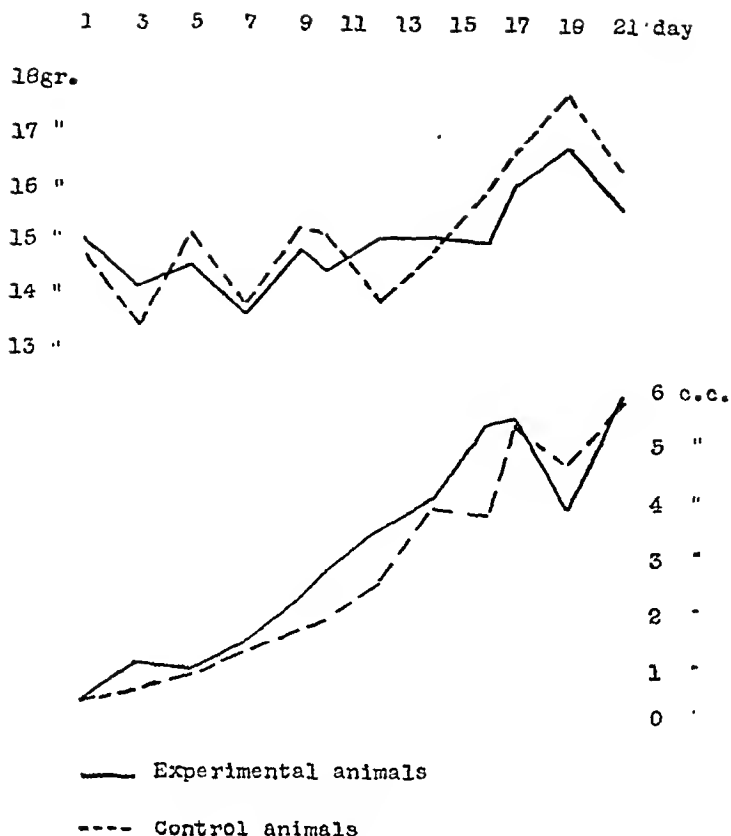


Fig. 2.

oil injected intra-abdominally, whereas the control animals received 0.1 c.c. olive oil.

The animals were weighed and their tumours measured daily or every second day. They were all killed 21 days after the injection.

The average weight and the average tumour size are shown together in Table 2 and Fig. 2.

As Table and Fig. 2 show, there was practically no difference between the average weights and the average tumour sizes of the two series. If there was any difference at all, the tumours of the experimental animals were if anything somewhat larger than those of the controls. The animals were

Table 3.

Average	Experimental animals		Controls		Weight-controls	
	Weight gr.	Measm't c. c.	Weight gr.	Measm't c. c.	Weight gr.	Measm't c. c.
Injection day	22.3	0.02	21.7	0.02	22.6	0.08
2nd day	19.4	0.02	20.4	0.02	20.5	0.09
3rd day	18.7	0.02	20.6	0.02	20.9	0.09
4th day	18.4	0.03	19.4	0.05	18.5	0.09
5th day	18.0	0.03	19.4	0.06	20.5	0.09
6th day	17.1	0.03	19.2	0.11	19.5	0.09
7th day	16.9	0.06	19.0	0.14	18.0	0.09
8th day	19.1	0.17	20.0	0.29	16.7	0.21
9th day	19.6		20.8		18.1	
10th day	19.1		21.5		17.3	
11th day	19.0	0.35	20.9	0.39	19.7	0.53
12th day	19.9	0.33	21.7	0.57	20.5	0.44
14th day	19.1	0.36	21.6	0.84	18.5	0.42
15th day	19.1	0.43	21.8	1.03	18.5	0.47
16th day	19.1	0.57	21.0	1.11	16.0	0.51
17th day	20.2	0.62	21.7	1.70	20.3	0.66
18th day	20.4	0.88	22.7	2.02	20.8	1.18
19th day	20.4	1.09	22.4	2.22	20.1	1.25

not affected by the injection, and clinically there was no difference between the two; nor was there any to be observed post mortem, and the subsequent histological examination revealed nothing abnormal in the parenchymeous organs, and no difference in the tumours of the two series.

The dose employed in this experiment did not affect the growth of the tumours, nor did it have any toxic influence on the animals. On comparing this result with that of the foregoing experiment one might be disposed to draw the somewhat hasty conclusion that the inhibitory effect is not a specific one, but due exclusively to the toxic action of the agent on the entire organism, manifested first and foremost by loss of weight. In the next experiments, in order to eliminate any error due to loss of weight, we introduced an extra control series in which the weight of the animals was caused to fol-

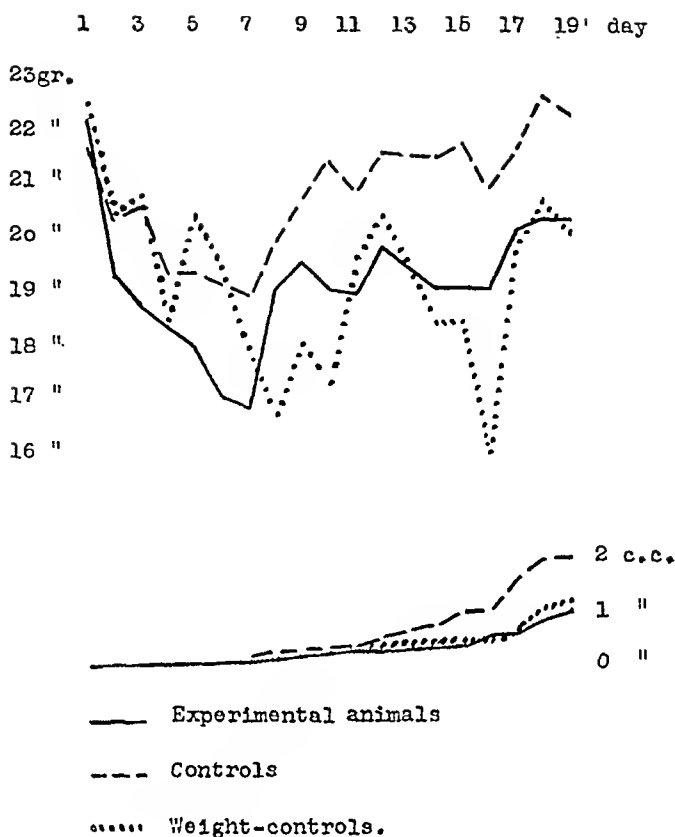


Fig. 3.

low that of the experimental animals as closely as possible by restricting the food of the former. In the following these extra-controls will be referred to as weight-control animals.

A total of 29 Dlb. mice were planted with a spontaneous mammary carcinoma from another Dlb. mouse. On the 9th day the experimental animals were given 1 mg. 9:10-dimethyl-1:2-benzanthracen dissolved in 0.1 c.c. olive oil, injected intra-abdominally. The controls and the weight-controls were given 0.1 c.c. olive oil. The animals were weighed and their tumours measured daily. Twenty days after the injection they were killed. Their average weight and the average size of the tumours are shown together in Table 3 and Fig. 3.

It will be seen from this table and figure that the experimental animals lost 5 gr. on an average in the course of the first week, but after that the weight increased, but at no time reached the level of the controls, whose weight was about 2 gr. more all the time.

The tumours of both experimental and weight-control animals grew more slowly than those of the controls. There was practically no difference between the tumours of the experimental and the weight-control animals; in any case the difference was so slight that no significance can be attached to it.

Throughout the experiment the experimental animals and those of the weight-control series were in some poor condition. Autopsy revealed nothing abnormal apart from the tumours, but here again the histological examination discovered slight parenchymatous degeneration of the liver. There was no difference in the tumours of the three series.

The result of this experiment was that 9:10-dimethyl-1:2-benzanthracene cannot be said to have any specific effect on the transplanted tumour, as we obtained practically the same inhibition of the tumour growth in the weight-control animals, whose weight was kept down by starvation, whereas the weight of the experimental animals fell because the agent affected them toxically, as was confirmed by the histological examination.

Crocker Sarcoma 180 in Strain Street.

Crocker sarcoma 180 was transplanted to 50 Street mice. On the 11th day the experimental animals were injected intra-abdominally with 1 mg. 9:10-dimethyl-1:2-benzanthracene dissolved in 0.1 c.c. olive oil. The controls and the weight-controls were given 0.1 c.c. olive oil. The animals were weighed and their tumours were measured daily. Thirty days after transplantation all the animals were killed. Their average weight and the average size of the tumours are shown together in Table 4 and Fig. 4.

Table 4.

	Experimental animals		Controls		Weight controls	
	Weight gr.	Measm't c. c.	Weight gr.	Measm't c. c.	Weight gr.	Measm't c. c.
1th day (injection day)	25.1	0.5	25.5	0.6	24.6	0.4
3th day	24.1	0.9	23.8	1.0	23.1	0.6
4th day	23.3	0.9	25.5	0.9	23.1	1.1
5th day	23.3	1.3	25.9	1.6	24.1	1.2
6th day	23.5	1.5	25.3	1.6	20.2	1.7
7th day	24.1	1.5	26.2	1.9	19.2	1.5
8th day	23.0	1.8	25.4	2.2	21.4	1.8
10th day	23.9	2.1	25.0	2.7	20.6	2.4
11st day	25.2	2.1	25.8	2.8	21.6	2.9
12nd day	27.3	2.6	26.4	3.2	23.3	3.1
13rd day	27.9	2.5	27.0	3.2	23.4	2.7
14th day	27.2	2.3	30.6	3.5	25.0	3.1
15th day	26.4	2.7	25.6	4.0	23.6	3.8
17th day	26.9	3.3	26.4	4.0	24.2	4.2
18th day	27.1	3.6	26.4	4.4	25.1	4.8
19th day	30.0	5.0	27.4	6.1	24.9	5.8
20th day	28.5	4.8	27.9	6.3	25.9	6.0

From Table 4 and Fig. 4 it appears that the experimental mice lost an average of about 2 gr. during the first four days, whereafter the weight was fairly stationary until the 9th day after the injection, whereafter it increased steadily, so that during the last five days of the experiment it was in fact over that of the controls. During the first five days the weight of the weight-control mice was almost the same as that of the controls, but after that the average was a good 3 gr. under theirs. The tumours grew gradually in all the animals; in the last ten days of the experiment, however, the tumours of the control mice were about $\frac{3}{4}$ c.c. less than those of the weight-controls.

The experimental animals were not particularly affected by the injections, nor were they distinctly poorer in condition than the weight-controls. Antopsy revealed ascites in five of

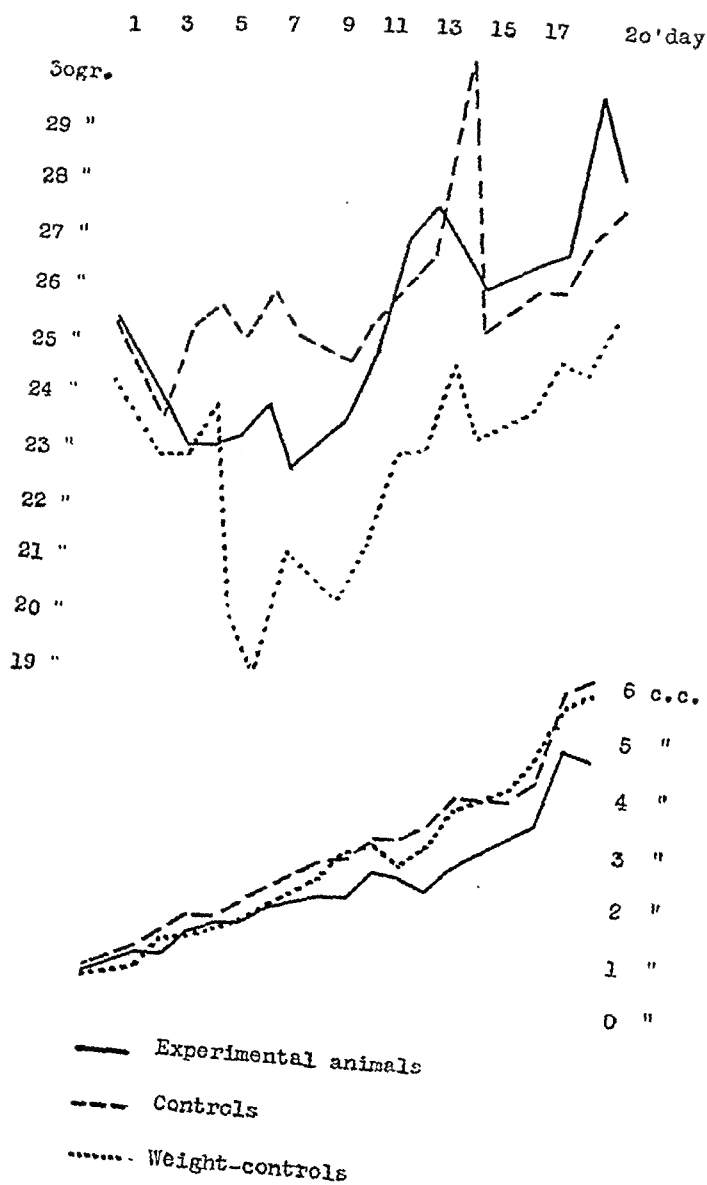


Fig. 4.

the experimental mice, a pale fluid in the peritoneum of four animals, one of which also had fibrinous coatings on the organs. In two there was sanguinolent fluid in the peritoneum,

and finally, nothing abnormal was seen in the peritoneum of four animals. The control and weight-control mice had no peritoneal changes. The histological examination revealed no differences in the tumours of the three series, but there were signs of slight parenchymatous degeneration in the liver of the injected mice. The result of this experiment was that 9:10-dimethyl-1:2-benzanthracene had a slight inhibitory effect on the growth of the Crocker sarcoma. Among the weight-controls the sarcoma did not seem to be much affected by starvation, for the average size of the tumours of the controls and the weight-controls corresponded almost throughout the experiment; the greatest difference to each side was — 0.5 c.c. and — 0.4 c.c.

In this case it cannot have been the loss of weight of the experimental animals that caused their tumours to grow less than those of the controls, as the tumours of the weight-controls grew just as much as those of the controls, though their weight throughout the greater part of the experiment was over 3 gr. less than that of the experimental animals.

It cannot be seen from this experiment whether the inhibition of the tumour growth in the experimental animals is due to a specific action by 9:10-dimethyl-1:2-benzanthracene, or it is merely the result of its toxic effect on the organism. In order to try to throw more light on this question the product in the following experiment was administered in such a small dose that it would have no toxic effect.

Crocker sarcoma 180 was transplanted to 48 Street mice. On the sixth day the experimental animals received 0.1 mg. 9:10-dimethyl-1:2-benzanthracene dissolved in 0.1 c.c. olive oil injected intra-abdominally. The controls and weight-controls received 0.1 c.c. olive oil. The animals were weighed and the tumours measured every other day. In Table 5 and Fig. 5 the average weight and the average size of the tumours are shown together.

None of the animals was affected by the injections, the weight curves did not vary much, and the tumours grew almost equally in all three series. Apart from the tumours,

Table 5.

Table 5.										
	Experimental animals		Controls		Weight controls					
	Weight gr.	Measm't c. c.	Weight gr.	Measm't c. c.	Weight gr.	Measm't c. c.				
1th day (injection day)	18.4		18.1		18.2					
3th day	16.4		15.7		16.6					
7th day	18.9		18.2		18.1					
9th day	16.8	0.9	17.5	1.0	17.2	0.7				
11th day	18.0	1.4	19.0	1.7	18.4	1.0				
13th day	17.5	2.0	18.8	2.0	17.0	1.6				
16th day	19.3	2.1	20.0	2.7	18.9	2.3				
18th day	16.8	2.8	18.1	2.9	16.9	2.5				
20th day	17.8	3.0	17.9	2.7	17.1	2.7				
	1	5	5	7	9	11	13	15	17	20th day

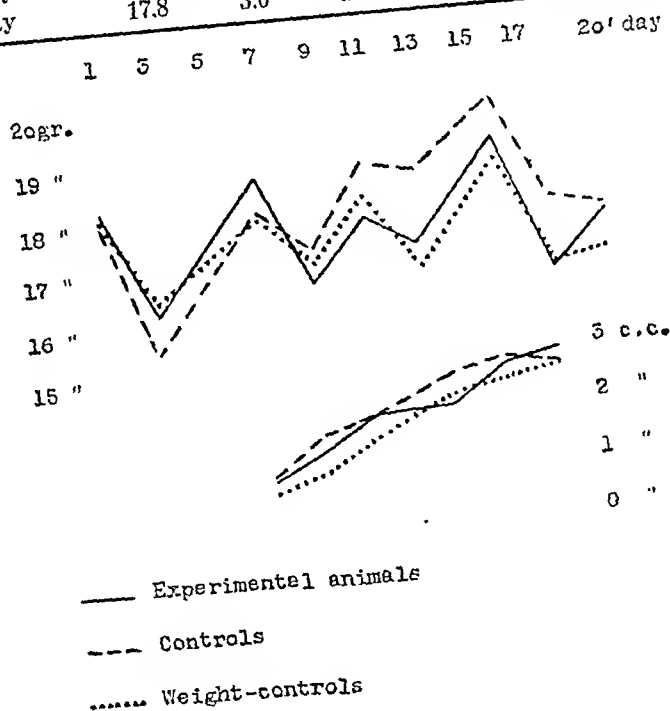


Fig. 5.

nothing abnormal was found at autopsy, and the histological examination showed no difference in the tumours of the three

Table 6.

	Experimental animals		Controls		Weight controls	
	Weight gr.	Measm't c. c.	Weight gr.	Measm't c. c.	Weight gr.	Measm't c. c.
1st day	15.3		16.2		17.0	
3rd day	15.3		17.0		17.8	
5th day	16.5		17.5		17.6	
7th day	15.7		17.1		15.8	
9th day	14.8		16.6		17.8	
10th day	16.4		19.0		16.5	
(Last inj. day)						
11th day	16.0		21.1		14.9	
12th day	13.8	1.2	19.8	2.6	13.6	2.02
13th day	14.7	1.6	19.4	3.4	15.4	2.10
14th day	12.5	1.5	19.4	3.6	16.8	2.40
15th day	14.1	1.5	20.5	5.8	13.4	2.50
16th day	14.5	1.8	20.6	6.9	13.6	2.90
17th day	14.3	1.4	20.3	6.9	11.0	3.10

series. The result of this experiment is that 9:10-dimethyl-1:2-benzanthracene does not inhibit the Crocker sarcoma when administered in such small doses. An examination was thereupon made of the effect of daily injections of this hydrocarbon. Crocker sarcoma was transplanted to 29 Street mice. During the next ten days the experimental animals received 0.1 mg. 9:10-dimethyl-1:2-benzanthracene dissolved in olive oil and injected intra-abdominally. The controls and the weight-controls received 0.1 c.c. olive oil.

The experimental animals became lower in condition with every injection; they lost weight and were tousled and cold. The weight-control animals, which were rigorously starved in order that their average weight might keep pace with that of the experimental mice, likewise lost condition. The animals in both series died quickly after the injections were discontinued, and the last experimental animal died 11 days after the last injection. At this time only one weight-control mouse was alive, whereas all the controls except one were living, the



— Experimental animals
 --- Controls
 Weight-controls

Fig. 6.

one having died the day after the last injection owing to enteritis. The average weight of the animals and the average size of the tumours are shown together in Table 6 and Fig. 6, which give the average of the animals living on the 17th day after transplantation (3 experimental animals, 4 weight-controls and 8 controls).

It is clear from Table 6 and Fig. 6 that after the injections were discontinued, the difference between the weight of the control animals on the one hand and that of the experimental and the weight-control animals on the other became greater and greater. Throughout the whole of the experiment the weights of the two latter series kept fairly close together. It will also be seen that there was an enormous difference between the tumours of the control animals on the one hand and those of the weight-controls and the experimental animals on the other. Furthermore, there was a rather marked difference between the tumours of the experimental animals and those of the weight controls, the latter's being almost twice as large. Oil was found post mortem in the peritoneal cavity of all the animals, but in the experimental animals there was also pale peritoneal fluid and, in several cases, fibrinous coatings on the organs. The histological examination revealed parenchymatous degeneration in the liver of the experimental animals. There was no difference in the histological picture of the tumours of the three series. The result of this experiment is that *9:10-dimethyl-1:2-benzanthracene* had an inhibitory effect on the growth of the tumour, as in the experimental animals the tumours were no more than just over half the size of those of the weight-controls, whose weight all the time was approximately the same as that of the experimental animals. The loss of weight and the histological examination show that the agent was very toxic in its effect.

On comparing the tumours of the weight-control animals in this experiment with those of the weight-controls in the first one, it will be observed that there is a great difference in the susceptibility of the tumours to inanition, for in the first experiment they were unaffected, whereas in the last

one they were greatly so. The explanation of this may be that in the first experiment the animals were not starved until the eleventh day after transplantation, that is to say after the tumour had »taken root«, whereas in the last experiment the animals were deprived of food from the day of transplantation. Experiments supporting this assumption were made by Raus (1914). Summarizing the results of these three experiments it may be said that 9:10-dimethyl-1:2-benzanthracene has a slightly inhibitory effect on the growth of Crocker sarcoma 180, but only when administered in toxic quantities.

Discussion.

Since Haddow published the first experiments in 1935, purporting to show that carcinogenous hydrocarbons have an inhibitory effect on malignant tumours, many workers have occupied themselves with the problem, which to all appearances is quite paradoxical. However, it is no unparalleled event that the same influence may cause cancer and also inhibit existing cancerous growths. X-rays and radium, which are carcinogenous in certain doses, can cure cancer, and therefore it was very natural to examine the question of whether the carcinogenous hydrocarbons also had an inhibitory, and perhaps a healing effect on cancerous tumours.

If one regards the cells of malignant tumours as cells that are defective in one or several respects, one may quite well imagine that the influence of carcinogenous hydrocarbons, which inhibit certain cell functions and may be contributory in the transformation of normal cells into tumour cells may also be capable of inhibiting the function of these cells and perhaps kill them.

In the publications referred to above it is stated by some workers (i.a. Haddow) that the growth of the experimental animals was retarded, sometimes so much that they even lost weight. As will appear from the experiments described above, this is a very important factor to be considered when

appraising the various experiments, but the various authors have not taken it up for closer examination. If one would measure the inhibitory effect of a substance on malignant tumours, and one knows in advance that this substance retards physical growth and may even cause the animals to lose weight, one must for every experiment have a control series whose weight is made to follow that of the experimental animals as closely as possible. Only in this manner is it possible to eliminate the error which may be comprised in the fact the growth of the experimental animals, and with it the growth of a transplanted tumour, is inhibited. Several investigations have been published on the influence of changes in physical growth on the growth of tumour tissue in the same organism.

Moreschi (1909) transplanted »Sarcoma 7« to mice weighing about 11 gr. The animals were thereafter divided into four groups, and these were given 1 gr., $1\frac{1}{2}$ gr. and 2 gr. of food daily, whilst the fourth group received food ad libitum. It was then found that the tumours grew in proportion to the amount of food eaten by the animals. The gross weight of the animals (body weight + tumour weight) was in direct ratio to the amount of food, falling with under-feeding and rising with over-feeding. It was also found that the net weight (body weight — tumour weight) always decreased, mostly with under-feeding but still to some extent with over-feeding. The apparent increase of total weight with over-feeding was due solely to the fact that the tumours in these cases grew most. Finally, it was shown that in many cases the under-fed animals died later than the over-fed ones. The experiments were repeated with animals weighing 20 gr., with the same result.

C. O. Jensen (1909) and Haaland (1907) each arrived at a similar result. Rous (1910 — 1911) found that Flexner-Jobling rat carcinoma did not take readily if the animals were starved prior to transplantation. If he starved the animals after the transplantation was established, there was no effect on the tumours. In contrast, Jensen's rat sarcoma was

very susceptible to starvation, even after it had grown for some time.

Mendel, Osborne and Ferry (1912) performed many experiments for the purpose of examining the influence of diet on body weight. In these they employed a diet consisting of purely vegetable proteins, on which the experimental animals did not grow; their weight remained stationary, but otherwise the animals seemed to be normal in every respect. Sweet, Corson-White and Saxon (1913) in their experiments tried a similar diet, with the same result. After the animals had been on this diet for fourteen days a carcinoma was transplanted (the Rockefeller Institute's mouse carcinoma No. 33) it took in all the control animals, but in only 9 of the 25 experimental animals. Twenty days afterwards five of these tumours had completely disappeared, and thirty days after transplantation two more. One of the remaining two animals died 52 days after transplantation with a tumour of 5.2 mm., and the same day the last mouse was put on normal diet, whereafter its tumour began to grow rapidly. Sixteen days after the transplantation the control animals were divided into two groups, one being given Mendel-Osborne's diet; the tumours of these latter animals grew only from 2.2 to 5.1 mm. on an average in the course of sixteen days, whereas those of the controls proper grew from 2.2 to 19 mm. in the same period. The experiment was repeated with Flexner-Jobling rat carcinoma and gave the same result. Rous (1914) showed that transplanted carcinomata grew indifferently in Japanese dancing mice and Strain 63 from the Imperial Cancer Research Fund when the animals lived on Sweet's modification of Mendel-Osborne's diet, i.e. when their growth was greatly inhibited.

All these experiments show with great clarity that if the experimental animals are under-fed, so that their growth is retarded or stopped, the growth of most transplanted tumours will also be retarded. There are several examples to show that the inhibitory effect on the growth of the tumour is not merely growth inhibition as a consequence of inanition, but

also as a consequence of many other influences on the animals. For instance, Mc. Euen and Thompson (1933) transplanted Walker carcinoma on rats, and fourteen days later removed their hypophysis. The tumours grew more slowly on the hypophysectomized animals, whose rate of growth was reduced considerably. In a control experiment some animals which had also been planted with Walker carcinoma were starved, and it was found that the growth of the tumours was retarded just as much as those of the hypophysectomized animals.

An experiment of similar character was carried out by Bishoff, Maxwell and Ullmann (1932), who transplanted rat sarcoma R 10, rat sarcoma 256 and mouse sarcoma 180, and also tested mice with spontaneous mammary carcinoma. After the transplantations the hypophysis of the rats and mice was X-rayed, and it was then seen that when the irradiation had been so strong that the physical growth of the animals was retarded, the growth of the tumours was also retarded. When the physical growth was not affected, the tumours were not affected in their growth either.

Haddow, Scott and Scott (1937) made experiments intending to show the effects of the carcinogenous hydrocarbons on physical growth. It turned out that rats treated with 10 mg. 1 : 2 : 5 : 6-dibenzanthracene had a reduced physical growth of up to 50 % in the course of 14 days. The normal rate of growth was not re-established in the fifteen weeks over which the experiment extended. If the doses of carcinogenous hydrocarbon were increased, the reduction of the rate of growth became still greater, and conversely, if only 3 gr. of the agent was given, there was also a reduction, though only a small one. By means of experiments with other carcinogenous hydrocarbons it was found that the effect on physical growth is not proportional to the carcinogenous activity of the agents, as 1 : 2 : 5 : 6-dibenzacridine (doubtful carcinogenous activity) caused a greater reduction of the rate of growth than a carcinogenous hydrocarbon so powerful as 3 : 4-benzpyrene. The experiments thus showed that the rate of growth of rats

is reduced, and may even cease, after the injection of carcinogenous hydrocarbons. The inhibition of the growth is of very long duration, the normal rate of growth never being re-established.

These experiments seem to support the opinion arrived at by Pollia (1937). He writes that the inhibitory effect of 1:2:5:6-dibenzanthracene on tumour growth, at any rate for the most part must be due to a constitutional lesion, and that the effect of this agent is not directed specifically against cancer. Meantime, Haddow, Scott and Scott maintained that the carcinogenous hydrocarbons had no toxic effect on the animals, and tried to prove their assertion by treating rats with various media like X-rays, plumbic nitrate, phenobarbitone and colchicine. However, they achieved nothing more than to inhibit the physical growth when the agents were administered in toxic doses. The authors concluded that in their experiments the carcinogenous hydrocarbons were not toxic to the animals, but the observations scarcely permit of that conclusion. The simple fact that an agent reduces physical growth and perhaps stops it is, for media such as those employed, sufficient to characterize them as toxic to the organism. Furthermore, several authors have demonstrated degenerative changes in the parenchymatous organs in animals treated with carcinogenous hydrocarbons.

Pollia found degenerative changes in the liver, Pybus and Miller in the liver and kidneys. Polson (1936) observed necrotic changes in the liver, and many other workers have found similar lesions (Maisin and Liegeois, 1934, Maisin and Coolen 1934, Picard and Laduron 1934, etc.). All these changes show distinctly that the carcinogenous hydrocarbons do not leave the organism intact and they better explain the growth inhibition that takes place.

Even if Haddow, Scott and Scott consider that the carcinogenous hydrocarbons are not toxic in their effect on the animals, neither they nor the other authors can draw any conclusions as to their effect on malignant tumours in experiments where the physical growth of the animals is re-

tarded by the treatment. The aforesaid experiments on the influence of physical growth on tumour growth show that physical growth has a decisive influence on the growth of most tumours, in any case on many of the tumours employed in the experiments with carcinogenous hydrocarbons. For instance, Haddow, and Haddow and Robinson in their work employed the very same tumours (Jensen's rat sarcoma, Walker carcinoma and Crocker sarcoma 180) as those used by Rous, McEuen and Thomson, and Bishoff, Maxwell and Ullmann, that is to say tumours that have proved to be highly dependent on physical growth.

As was stated above, Haddow, Scott and Scott found that there was no parallelism between the activity of the carcinogenous hydrocarbons and their inhibitory effect, nor was there any parallelism between their carcinogenous activity and their influence on physical growth. It would be interesting to have it examined whether the media having the strongest inhibitory effect on physical growth do not inhibit tumour growth most as well. One example to show that this may be so is to be had by comparing various experimental results. 1:2:5:6-dibenzaeridine, which has a very faint carcinogenous activity, in Haddow and Robinson's experiments (1939) proved to have a strong inhibitory effect on spontaneous mouse carcinomata and also on Crocker sarcoma 180. The inhibition was more marked than that of a carcinogenous hydrocarbon so powerful as 3:4-benzpyrene. In experiments by Haddow, Scott and Scott (1937) it was found that 1:2:5:6-dibenzaeridin reduced the physical growth of rats much more than 3:4-benzpyrene did.

It has already been stated that the inhibitory effect is not confined to the carcinogenous activity of the hydrocarbons (Haddow and Robinson 1939). Hydrocarbons closely related to the carcinogenous varieties but with no carcinogenous activity have often proved to have an inhibitory effect on tumour growth. Unfortunately, nothing is stated as to whether they simultaneously inhibited physical growth; but after what

has been said above, it is presumable that they did have this effect.

This being so, the results of all previous inhibition experiments with carcinogenous hydrocarbons must be viewed with the greatest reserve. It must again be pointed out that no conclusion can be drawn from comparisons between experimental animals and control animals if their weights diverge as a consequence of the effects of the treatment on the growth of the experimental animals. It is only when the experimental animals are compared with animals kept at the same weight that the effect of the medium can really be judged.

Turning now to an appraisal of own experimental results on the basis of this discussion it may be said that 9:10-dimethyl-1:2-benzanthracene undoubtedly has an inhibitory effect on Crocker sarcoma 180. It appears from Fig. 6 and Fig. 4 — the latter to a lesser degree — that the tumours of the experimental animals were distinctly slower in their growth than those of the weight-control animals, i.e. they were smaller than those of the control animals, which throughout the whole experiment weighed the same as, and in many cases less than, the experimental animals.

The 9:10-dimethyl-1:2-benzanthracene had no observable effect on the transplanted mammary carcinoma on Dlb. mice. Unfortunately, as a consequence of unsatisfactory breeding in the Dlb. strain it has not yet been possible to undertake experiments with the medium administered in small daily doses, the method which in experiments with the Street mice caused the most pronounced inhibition.

Summary.

After a brief survey of earlier works on the inhibitory effect of carcinogenous hydrocarbons on malignant tumours, a description is given of own experiments with 9:10-dimethyl-1:2-benzanthracene on transplanted mammary carcinomata

in the Dlb-strain and transplanted Crocker sarcoma 180 in the Street strain.

The medium was injected intra-abdominally, dissolved in olive oil. After the first experiment it appeared that the experimental animals lost weight as a result of the treatment. In order to eliminate the possible error involved in this factor, an additional control series was introduced into the subsequent experiments, called »weight-control animals«, whose weight was kept as near to that of the experimental animals as possible by reducing their food.

In the next experiments it was found that 9:10-dimethyl-1:2-benzanthracene had no inhibitory effect on the transplanted mammary carcinomata in strain Dlb., but a distinct effect on Crocker sarcoma 180 in the Street strain, but only when the medium was given in toxic doses.

The influence of loss of weight on the growth of malignant tumour is further examined in the discussion, and a warning is uttered against drawing too far-reaching conclusions from experiments in which the experimental animals are compared only with controls whose weight throughout the experiment is higher than that of the experimental animals.

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THE HISTOPATHOLOGY OF THE ALLERGIC SKIN REACTIONS.

By *Egon Bruun*.

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Allergic skin reactions can be elicited in different ways: Partly, cutaneously as excoriation tests, partly, intracutaneously as blistering tests, and finally, epicutaneously as patch tests. This latter mode of application, the technic of which was devised by *Bruno Bloch*²), has proved very significant and advantageous in dermatology, where patch tests are ever more frequently resorted to as a decisive diagnosticum in allergic skin affections.

On the ground of the time elapsing till a positive reaction becomes visible, the allergic skin reactions may be divided into instantaneous reactions and delayed reactions.

Patch reactions will always appear as delayed reactions, since a positive reaction will require at least 24 hours and, sometimes, 48 → 72 hours, before it becomes visible. Quite exceptionally only will a patch test be responded to by positive reaction within a shorter space of time, for example 7 hours, and never so rapidly as an instantaneous reaction.

Instantaneous reactions can be carried out as excoriating tests or as blistering tests, in either case the protective action of the stratum corneum is eliminated, and the allergen applied is enabled to react almost instantaneously with the antibodies bound to the parenchymatous cells of the skin. A

positive reaction will appear almost instantaneously, at any rate within 20 minutes.

Thus, excoriating tests and intracutaneous tests *may* elicit instantaneous reactions but not necessarily; for example, neither Pirquet's reaction nor Mantoux' reaction becomes positive till at least 24 hours have elapsed. Patch tests on the contrary, as mentioned above, always produce delayed reactions.

The allergic skin reactions may also be divided according to their clinicomorphological mode of reaction. W. *Jadassohn*⁸) divides them into (1) urticarious, (2), eczematous and, (3) neither urticarious nor eczematous, forms of reaction. *Jaffé*¹⁰) closely follows this author and likewise divides the reactions into 3 groups:

(1) The allergic skin reaction presents itself as an urticarious reaction chiefly consisting of exudative-vascular changes. This type of reaction is particularly met with in patients with asthma, urticaria, allergic rhinitis and conjunctivitis, allergic prurigo (Besnier), hemicrania and in many rheumatics.

(2) The allergic skin reaction presents itself as an eczematous reaction chiefly consisting of epithelial changes. This type of reaction is observed in patients with allergic eczema.

(3) The allergic reaction presents itself as an infiltrative process in the skin chiefly consisting of cellular and vascular changes. According to *Jaffé* this type may be observed in reactions opposite bacterial antigens.

Just as in all other biological reactions there are gliding transitions between the 3 types of reaction.

Whether a positive reaction be intended as instantaneous or as delayed reaction, it represents an acute allergic inflammation in the skin. The principle in allergic reaction is the phenomenon of Arthus. The pathological anatomy in the phenomenon of Arthus was described by *Gerlach*⁶) in a great work: *Die hyperergische Entzündung*, issued in 1923.

Gerlach describes the details of the phenomenon of Arthus, which he elicited in rabbits, guinea-pigs, dogs, and Man, partly with hog serum, partly with horse serum, and he shows that the

mode of reaction of animals of different species may vary in degree but not in principle. The histological characteristic of Arthus's phenomenon is central necrosis due to stasis, edema, tissue swelling, and fibrinoid exudation, encircled by very pronounced cellular infiltration. In the majority of animal species the central processes led to necrosis, only in rats was the reaction less pronounced in degree, edema and tissue swelling being observed but no necrosis; peripherally was observed cellular infiltration as in other species of animals. The question whether the phenomenon of Arthus can be elicited in rats thus becomes a problem of definition. *Longcope*¹³⁾ and *Opie*¹⁵⁾ are of opinion that Arthus's phenomenon cannot be elicited in rats. If central necrosis is claimed as an indispensable characteristic of Arthus's phenomenon, this phenomenon certainly cannot be elicited in white rats; however, that the changes produced in the tissues are allergic tissue processes, is beyond all doubt.

The cellular infiltration encircling the central necrosis in all the experiments performed by *Gerlach* consisted, in the acute stage, of polymorphonuclear leukocytes. Not till 34 hours at least after the injection did rabbits present incipient growth of adventitial and histiogenous cells, and after 51 hours the polymorphonuclear leukocytic cell element still had the preponderance. After 61 hours the mobilized connective tissue cells had become numerous.

Thus the histological characteristic of Arthus's phenomenon is a central necrosis encircled peripherally by a strong cellular infiltration, which practically consists exclusively of polymorphonuclear leukocytes. Comparatively soon the process shows signs of passing into a subacute stage with ingrowth of histiogenous cells. If the allergic reaction is not strong enough to cause necrosis, it is called hyperergic inflammation. In case of hyperergic inflammation — described by *Rössle*¹⁷⁾ in 1914 — stasis, edema, tissue swelling, fibrinoid exudation, leukocytic infiltration with many eosinophils are observed but no necrosis. Arthus's phenomenon must therefore be interpreted as the pathologico-anatomical climax of the hyperergic inflammation. Also in the reports of the histopathology of allergic skin reactions the characteristic feature of this form of acute allergic inflammation is described as a chiefly, or purely, polymorphonuclear leukocytic reaction. Among the polymorphonuclear leukocytes may be found a very great number of eosinophils. *Berger & Lang*¹⁾ in an allergic sensitive to flour examined the histological reaction from $\frac{1}{2}$ hour to 24 hours after application of the specific allergen. They made use of *intracutaneous tests* and, at the 7 hour stage, observed violent reaction up to abscess formation in corium and subcutis, presenting almost

exclusively polymorphonuclear leukocytes 50 per cent of which were eosinophils. There was lively disintegration of cells with tissue necrosis. After 24 hours the eosinophilia decreased somewhat, and a touch of lymphoid cells was observed though with distinct preponderance of polymorphonuclear leukocytes.

Jadassohn⁹⁾ corroborated Berger & Lang's observations but reported at the same time that morphine vesicles, atrophine vesicles, pilocarpine vesicles, and vesicles of mechanical origin in normal individuals likewise present distinct eosinophilic reaction. Jadassohn therefore does not consider eosinophilia as a sign of an allergic reaction but as a sign of an urticarial mode of reaction.

Miescher¹⁴⁾ examined the histological picture with the aid of patch tests on allergics, and compared the tissue reaction with the histological picture which appears in the skin of normal persons after application of a toxic irritant (for example mustard plaster, croton oil, and hydrochloric acid). In typical toxic reactions areas of inflammatory necrosis with sharp outlines are observed; even very extensive necrotic inflammations heal comparatively rapidly. Toxic inflammatory reaction never is attended by tissue eosinophilia. In allergic eczema tests, i.e. patch tests, typical cases present status spongiosus with intraparenchymatous vesicle formation in the epidermis as well as tissue eosinophilia. However, status spongiosus may also be met with in case of toxic reaction, for example to croton oil, and the tissue eosinophilia varies. Thus Miescher found strong tissue eosinophilia in patients who were hypersensitive to panthesin liniment, whereas positive reactions after application of salvarsan and balsam of Peru never were attended by local tissue eosinophilia. Miescher therefore concludes that the clinical picture of patch tests and the microscopically detectable spongiosis in the epidermis are characteristic but not pathognomonic of allergic eczema, as they may also be observed after application of purely toxic substances. Tissue eosinophilia is suggestive of allergy but the absence of tissue eosinophilia does not speak against allergy. Nor did Miescher find quite regular conditions with regard to leukocytic infiltration in epidermis, corium and subcutis, both the allergic and the toxic patch tests revealing now preponderance of polymorphonuclear leukocytes, now of lymphocytes. Yet, in the allergic reactions the epidermal spongiosis presented a fairly constant preponderance of lymphocytes. In order to emphasize that most of the cells that have migrated into the epidermis are mononuclears Bonnevie³⁾ suggests supplementing the term *epidermitis spongiovesiculosa* by adding the adjective *lymphocytica*.

Klinge's¹¹⁾ investigation in acute anaphylactic arthritis in rab-

bits revealed an inflammation of almost purely polymorphonuclear leukocytic composition. In contradistinction to this *Bruun*⁴⁾, who copied *Klinge*'s experiments, found an acute allergic inflammation in the injected joint, where almost purely lympho-histioeytic cell infiltrates were observed in *all* the cases. Moreover, in many cases were found acute allergic myocardites of likewise lymphohistioeytic composition. The described processes were 24—48 hours old; in such cases *Rössle*, *Gerlach* and *Klinge* found polymorphonuclear leukocytic reaction. In this connection it shall be mentioned that *Bruun* did find all the signs of acute allergic inflammation (stasis, edema, tissue swelling, fibrinoid exudation) but never so strong a reaction as to cause necrosis. This may have different causes which must probably be sought in the serum applied. Thus *Klinge*¹²⁾ in one of his latest works declares that the *degree of the sensitization* may play a part for the histological mode of reaction: ».... Hier ist aber zu sagen, dass auch gleich am Anfang, beim Allergieversuch wie beim Menschen, diese Reaktion (i.e. the mono-histioeytic reaction) beherrschend sein kann. In manchen Fällen allergischer Urticariaquaddeln werden schon frühzeitig vorwiegend monozytäre Exsudate gefunden, und es mag vorwegnehmend darauf hingewiesen werden, dass das jeweilige zellige Entzündungsbild wie von der Art des Antigens, so auch von dem Grad der Sensibilisierung abhängt«.

*Page*¹⁶⁾, who has also carried out experiments of acute anaphylactic inflammation in rabbits and guinea-pigs described, besides the central necrosis encircled by leukocytic infiltration with many eosinophils, an infiltration of large mononuclears and macrophages in the periphery of the cellular infiltration as well as in the regions of tissue, which lie at a distance from the focus proper, and also an infiltration of mononuclear cells, which appears already during the acute stage (24 hours after the injection).

There is no doubt that *Gerlach* has hypersensitized his experimental animals, and thus produced very intensive reactions, and the fact that *Klinge* used fresh horse serum, whereas *Bruun* applied stored serum, has probably become the cause of manifestations of different reactivity. The primary tissue reaction in acute allergic inflammation consists of stasis, edema, swelling of the elementary substance with fibrinoid exudation. Next, the tissue is infiltrated with lympho-histioeytes, as was described, for example, in urticaria by *Török*, *Lehner* & *Kenedy*²⁰⁾. In the typical phenomenon of Arthus the vascular stasis and the edema are so dominant that necrosis ensues, and *Bruun*⁵⁾ thinks that the polymorphonuclear leukocytes do not appear till the necrotic processes have

formed, i. e. secondarily in relation to primary allergic tissue processes.

It must, however, be kept in mind that the phenomenon of Arthus is an artefact, which can only serve as a standard test of the mode of response of the tissues to particular artificial influences. With the aid of patch tests we get, however, much nearer the generally occurring («natural») morbid process in Man. In many cases, for example in patients with allergic eczema, the patient's eczema can be reproduced with patch tests in isolated places, where it can be followed from the very acutest stage and to the moment when it subsides entirely. By varying the concentration of the specific antigen applied as well as the time of application, a process can be elicited which appears perfectly identical to the patient's affection.

If a patient who works with chrome compounds incurs eczema and reacts positively to cutaneous application of chrome as antigen, it is scarcely dubious that he is an allergic responding with eczema to the influence of chrome. The diagnosis is substantiated, if he, after recovery, reincurs eczema when he resumes his work with chrome compounds.

Twenty patients who were treated in the dermatological department of the Rigshospital and who were ascertained to be allergics according to the afore-mentioned criterion, were tested cutaneously with the specific antigen; as soon as they presented positive reaction, the reacting part of the skin was submitted to biopsy and microscopy. To the tests were preferably submitted patients who reacted monospecifically, that is to say, to one single substance, and with regard to whom it was reliably ascertained that the substance under consideration was the moribific agent. A few of the patients certainly did respond to several patch tests (polyspecifically), but such patients were only included in the material, if they responded very strongly to that antigen which was incriminated for their disease, the other positive reactions at the same time being so faint that they must be considered

as »unspecific« in relation to the patient's skin disease. Moreover, the substances for the cutaneous reactions were always applied in such weak concentrations that a corrosive or other action, which would manifest itself also in non-allergics, was excluded. *Steiner*¹⁸⁾ for example has shown that a 3 per mille sublimate solution has an irritative effect on healthy skin, whence concentrations below 3 per mille ought to be used for allergy tests.

On those 20 patients biopsy of 22 cutaneous reactions was performed, 2 patients (Nrs. 12 and 13) being submitted to two tests each. The patients are divided into 3 groups:

(1) Thirteen patients (Nrs. 1—13, Scheme I) suffering from allergic eczema. The patch tests revealed that the eczematogenous substances were sublimate, balsam of Peru, turpentine, primula obconica, chrome, coal-tar, Cuba mahogany, and fir-wood.

(2) Four patients (Nrs. 14—17) were submitted to intracutaneous allergy tests which were of interest for their disease, 2 of them, suffering from epidermophytosis, being submitted to a trichophytin test, 1 patient with ulcer molle, to the Dmelcos test, and 1 patient with bronchial asthma and chronic bronchitis, to Mantoux's test.

(3) Three patients (Nrs. 18—20), who had been admitted to the department because of non-allergic affections (gonorrhea, scleroderma, non-allergic dermatitis), were sensitized to the action of dinitro-chlorbenzene after the method suggested by *Haxthausen*⁷⁾ and, afterwards, submitted to patch tests with dinitro-chlorbenzene.

The patch was mostly placed on the anterior and lateral surface of the upper third of the femur, where biopsy could be made under conduction anesthesia of the lateral cutaneous femoral nerve, which rendered it possible to anesthetize at some distance from the place of incision. The anesthetic applied was a novocain-adrenalin solution. Several of the patches were placed on the back between the scapulae, where biopsy

was made on an area encircled by a large rhomboid anesthesia induced at a convenient distance from the place of reaction.

The microscopy and the pathologico-anatomical description of the reactions were carried out by the prosectors of the pathologico-anatomical institute of the Rigshospital, because I thought it would be appropriate to obtain a »neutral« valuation. I wish to express my appreciation to the prosectors *V. Eskelund*, M. D., and *G. Teilum*, M. D., for their helpfulness in carrying out the microscopy of the many preparations.

For the differential counts on the preparations, which were made by the author, the strongest immersion lens was used, the cells in 5 different fields with pronounced reaction in each preparation being counted with a view to the number of polymorphonuclear leukocytes and lympho-histiocytic cell forms. The group of lympho-histiocytic cells chiefly comprises lymphocytes, large mononuclears and histiocytes but also plasma cells, macrophages, fibroblasts, and fibrocytes. Only cells lying extravascularly were included in the count, i. e. the tissue reaction proper, whereas cells doubtlessly lying intravascularly were omitted (see below). Percentual calculations of the counts were omitted intentionally. For that purpose too few cells were counted (fields with a comparatively small number of cells preferably being selected for the sake of perspicuity), and the counting technic is too uncertain, since the cells lie in several planes and many of them therefore may be overlooked. However, I think that with the method which I applied I have obtained a fairly correct estimate of the cellular composition of the reactions with regard to those two cell components that are of interest. It is a matter of course that the fields were not selected so that places which were typical for that cell reaction I »desired« to see were preferred, while other, more »atypical« places were discarded. That would also a priori be impossible, since the composition of the reactions was the same everywhere, as is evidenced by the descriptions supplied by the prosectors.

Biopsy was generally made 24 hours after application of the substance. Two (Nrs. 5 and 11, Scheme I) were made

after 48 hours, because the 24 hour reactions were not reliably positive, and one (Nr. 12 a) was made after the lapse of 7 hours. It would have been desirable, if biopsy had been made in a greater number of cases after the lapse of 7 hours, among other things because *Berger & Lang*, who worked with instantaneous reactions, made biopsies at the 7 hour stage. That was not feasible, however, because the patch tests generally were negative or, at the highest, very faintly positive at the 7 hour stage with slight redness but without eczematoid vesicles and infiltration. A single patient (Nr. 12), who presented positive reaction to a patch test after the lapse of 7 hours, was afterwards subjected to a 7 hours' blistering test.

Microscopy did not reveal any difference between the epicutaneous and the intracutaneous reaction (Nrs. 12 a and 12 b, Scheme I).

As signs of positive reaction to the patch tests were claimed redness and slight infiltration of the skin with formation of small vesicles, i. e. the usual eczema criteria. As in all biological reactions there were gliding transitions from relatively faint though distinct reactions to very strong reactions but never with formation of necrosis. There seemed to be no definite proportionality between the intensity of the reaction and the extension and degree of the eczema. A patient with an »innocent« and not very extensive eczema, for example on the hands, could very well experience a very strong cutaneous reaction; the reverse was of less frequent incidence, a patient with very intense allergic eczema as a rule presenting a strongly positive cutaneous reaction (in so far as it was possible to detect the eczematogenous agent).

The experimental results are recorded in Scheme I, which shows that 20 patients (10 men and 10 women) aged 15 to 71 years were submitted to allergic cutaneous tests, and that microscopy of pieces of tissue removed for biopsy from the cutaneous reactions revealed, *in all cases, an inflammation which, according to its manner of origin, must be considered as an acute allergic inflammation but in which the cell in-*

filtrates virtually present exclusively lympho-histiocytic cell reaction. Fig. 1 and Fig. 3 are typical pictures. The lympho-histiocytic preponderance was very convincing, only 0—→ 10 polymorphonuclear leukocytes being found among several hundred cells. The organism evidently had mobilized the polymorphonuclear leukocytes, for many such were

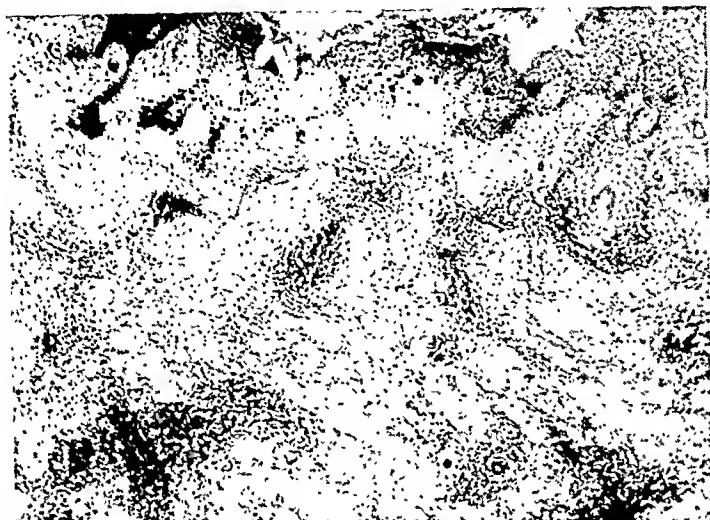


Fig. 1.

Case Nr. 5. Hématoxylin-eosin staining, enlarged about 120 times. Patch test with chrome 1 per mille. Biopsy after 24 hours. Upward, epidermis with sero-sanguineous exudation, beneath which, corium studded with cell infiltrates.

observed in the lumina of the vessels without, however, participating in the tissue reaction, for they were exceptionally only found extravascularly. This must probably be explained by the organism in case of an inflammatory process mobilizing all the available protective elements and sending them to the place of inflammation, where a suitable selection takes place. That the polymorphonuclear leukocytes thus move towards the place of inflammation without emerging from the vessels must be due to certain factors (negative chemotaxis?) preventing it; therefore it is warrantable to think that the polymorphonuclear leukocytes only will be attracted and emerge

Scheme I.

Pat. Nr.	Age (years)	Clinical diagnosis	Kind and standing of reaction	Differential count (5 fields altogether)	Microscopy
1	71	Allergic eczema	24 hours' patch test with sublimate 0.5 per mille	Among 348 cells were found 5 polymorphonuclear leukocytes and 343 lympho-histiocytic cells	The epithelium is edematous, lacerated, blurry; bad nucleus staining in several places; in a cavity in the very epithelium are found many lymphocytes and some polymorphonuclear leukocytes. The connective tissue beneath the epithelium densely infiltrated. No extravascular polymorphonuclear leukocytes. Round the lymph vessels of cutis conglomeration of lymphocytes. <i>Eskelund.</i>
2	43	Allergic eczema	24 hours' patch test with balsam of Peru	Among 304 cells were found 6 polymorphonuclear leukocytes and 298 lympho-histiocytic cells	Part of the epithelial surface is very edematously changed, with cavities containing lymphocytes and some polymorphonuclear leukocytes. The cavities in the epithelium apparently are due to direct exudation in the epithelium, and partly to strong edema in the papillae of the cutis. There are vacuoles quite down to the deepest layers of the epithelium. Around lymph tracts in the cutis there is moderate agglomeration of lymphocytes, whereas polymorphonuclear leukocytes fail to be seen. There is hyperemia of the capillaries corresponding to the epithelial changes, and the edema in the papillae extends somewhat downward in the cutis. <i>Eskelund.</i>
3	29	Allergic eczema	24 hours' patch test with turpentine	Among 582 cells were found 6 polymorphonuclear leukocytes and 576 lympho-histiocytic cells	In the connective tissue, particularly in the uppermost part, larger cellular infiltrates consisting almost exclusively of lymphocytes are seen. <i>Teitum.</i>

4	42	Allergic eczema	24 hours' patch test with extract of primula obconica	Among 325 cells were found 10 polymorphonuclear leukocytes and 315 lympho-histiocytic cells	The epithelium is well preserved, its surface being covered by erythrocytes. In the corium are seen scattered cellular infiltrations consisting almost exclusively of lymphocytes. <i>Teilum.</i>
5	26	Allergic eczema	24 hours' patch test with chrome 1 per mille	Among 395 cells were found 5 polymorphonuclear leukocytes and 390 lympho-histiocytic cells	There are fairly considerable cell infiltrates, particularly just beneath the epithelium. There are both lymphocytes and isolated polymorphonuclear leukocytes, the former however having the preponderance. <i>Teilum.</i>
6	56	Allergic eczema	24 hours' patch test with coal-tar	Among 323 cells were found 7 polymorphonuclear leukocytes and 316 lympho-histiocytic cells	In the subcutis is seen comparatively scanty cellular infiltration in oblong heaps. There is essentially a question of agglomerations of lymphocytic cells. <i>Teilum.</i>
7	19	Allergic eczema	24 hours' patch test with sublimate 0.5 per mille	Among 464 cells were found 2 polymorphonuclear leukocytes and 462 lympho-histiocytic cells	In the subcutis is seen fairly considerable cellular infiltration forming larger heaps. The reaction is almost exclusively lymphocytic. <i>Teilum.</i>
8	31	Allergic eczema	24 hours' patch test with Cuba mahogany	Among 241 cells were found 2 polymorphonuclear leukocytes and 239 lympho-histiocytic cells	There are remains of surface epithelium and, beneath it, moderate infiltration of lymphocytes around sweat glands and lymph vessels. No polymorphonuclear leukocytes are visible. <i>Eske Lund.</i>
9	15	Allergic eczema	24 hours' patch test with fir-wood	Among 311 cells were found 8 polymorphonuclear leukocytes and 303 lympho-histiocytic cells	Both in the epithelium and in the subcutis round the lymph tracts fairly diffuse infiltration with lymphocytes is seen. No polymorphonuclear leukocytes are observed outside the vessels. <i>Eske Lund.</i>

Scheme I (continued)

Pat. Nr.	Age (years)	Clinical diagnosis	Kind and standing of reaction	Differential count (5 fields altogether)	Microscopy
10	57	Allergic eczema	24 hours' patch test with turpentine	Among 312 cells were found 6 polymorphonuclear leukocytes and 306 lympho-histiocytic cells	Microscopic examination of the subcutis reveals a good many, partly scattered, partly perivascular, cells. The perivascular infiltrates consist quite predominantly of lymphocytes, and there are practically no extravascular polymorphonuclear leukocytes. Further are seen a few somewhat more scattered plasma cells and isolated eosinophilic leukocytes. <i>Estelund.</i>
11	22	Allergic eczema	48 hours' patch test with chrome 1 per mille	Among 349 cells were found 6 polymorphonuclear leukocytes and 343 lympho-histiocytic cells	There are moderate conglomerations of lymphocytes, particularly round the lymph tracts. <i>Estelund.</i>
12 a	52	Allergic eczema	7 hours' patch test with chrome 1 per mille	Among 494 cells were found 7 polymorphonuclear leukocytes and 487 lympho-histiocytic cells	In the subcutis are seen a good many cellular infiltrations consisting of lymphocytes. Eosinophilic cells are nowhere to be seen. <i>Teitum.</i>
12 b	52	Allergic eczema	7 hours' intracutaneous (vesicle) reaction with chrome 1 per mille	Among 432 cells were found 7 polymorphonuclear leukocytes and 425 lympho-histiocytic cells	In several places beneath the epithelium cellular conglomerations are seen, which everywhere consist almost exclusively of lymphocytes. No eosinophilic cells. <i>Teitum.</i>
13 a	32	Allergic eczema	24 hours' patch test with chrome 0.1 per mille	Among 331 cells were found 3 polymorphonuclear leukocytes and 328 lympho-histiocytic cells	Beneath the epithelium and in the subcutis, especially perivascularly, small lymphocytic infiltrations are seen. There are no polymorphonuclear leukocytes. <i>Teitum.</i>

13 b	32	Allergic eczema	24 hours' patch test with chrome 1 per mille	Among 466 cells were found 4 polymorphonuclear leukocytes and 462 lympho-histiocytic cells	Partly subepithelially, partly farther downward in the subcutis, oblong or round conglomerations of lymphocytes are seen. There are no polymorphonuclear leukocytes. <i>Teilum.</i>
14	19	Ulcus molle	Positive Dmelcos reaction; biopsy after 24 hours	Among 308 cells were found 2 polymorphonuclear leukocytes and 306 lympho-histiocytic cells	The cellular reaction in the subcutis is relatively little pronounced, and it consists of lymphocytes. There are no extravascular polymorphonuclear leukocytes, the changes, as was mentioned, being but little pronounced. <i>Estlund.</i>
15	34	Epidermophytosis	Positive trichophytin reaction; biopsy after 24 hours	Among 474 cells were found no polymorphonuclear leukocytes and 474 lympho-histiocytic cells	In the subcutis is found strong perivascular and somewhat more diffuse infiltration consisting exclusively of lymphocytes. There is some vascular epithelial degeneration and desquamation. <i>Estlund.</i>
16	39	Epidermophytosis	Positive trichophytin reaction; biopsy after 24 hours	Among 398 cells were found 5 polymorphonuclear leukocytes and 393 lympho-histiocytic cells	There is strong perivascular infiltration with lymphocytes. No polymorphonuclear leukocytes. <i>Estlund.</i>
17	46	Bronchial asthma, chronic bronchitis	Positive Mantoux 1:10000; biopsy after 24 hours	Among 378 cells were found 4 polymorphonuclear leukocytes and 374 lympho-histiocytic cells	In skin and subcutis are seen scattered infiltrates which consist chiefly of lymphocytes. <i>Estlund.</i>

Scheme I (continued)

Pat. Nr.	Age (years)	Clinical diagnosis	Kind and standing of reaction	Differential count (5 fields altogether)	Microscopy
18	50	Dermatitis	24 hours' patch test with dinitrochlorbenzene 1 per cent	Among 472 cells were found 6 polymorphonuclear leukocytes and 466 lympho-histiocytic cells	Microscopy reveals moderate conglomerations of lymphocytes in the cutis, particularly round the lymph tracts. Through the epithelium the infiltration reaches quite outward to the surface. There are practically no polymorphonuclear leukocytes. <i>Estlund.</i>
19	18	Gonorrhea	24 hours' patch test with dinitrochlorbenzene 1 per cent	Among 429 cells were found 7 polymorphonuclear leukocytes and 422 lympho-histiocytic cells	In the connective tissue, particularly round the lymph tracts and isolated capillaries, fairly considerable conglomerations of cells are seen, which quite predominantly consist of lymphocytes and of isolated plasma cells. In the vessels a good many polymorphonuclear leukocytes are seen but none extravasicularly. In the tissue, too, farther away from the vessels, scattered lymphocytes are seen. <i>Estlund.</i>
20	9	Scleroderma	24 hours' patch test with dinitrochlorbenzene 1 per cent	Among 278 cells were found 5 polymorphonuclear leukocytes and 273 lympho-histiocytic cells	Round the lymph tracts beneath the epidermis there is scanty infiltration with lymphocytes and isolated plasma cells. no polymorphonuclear leukocytes. <i>Estlund.</i>

from the vessels, if other factors (disintegration of cells with necrosis, bacteria) than purely allergic factors assert themselves. However, the reactions in the skin or subcutis were nowhere so intensive as to give rise to necrotic processes; nor was there any question of microbial infection.

On the other hand, a good many cases presented distinct epidermal disintegration with laceration of the epidermal surface and exudation of serosanguineous fluid, cell disintegration and superficial necrosis in those places, where the patch had been applied to the skin. In these places, i. e. epicutaneously, numerous polymorphonuclear leukocytes were seen in the inflammatory exudation, probably on account of cell disintegration with superficial necrosis; but it may also be thought that the bacteria, which always are present on the surface of the skin, may play a rôle for the emigration of the polymorphonuclear leukocytes to the epidermal surface.

As is evident from the description of the microscopy of the allergic skin reactions of Case 1 and Case 2, cavities with scanty content of cells of lymphocytic type were found in the epidermis. In order to avoid repetitions the description of the epidermis is omitted in the following, the interest being focussed exclusively on the subepidermal cell infiltrates the study of whose composition has been the special object of this work. However, the missing description of the epidermis must not be identified with the absence of epidermal cavities in the remaining cases. In by far the majority of the cases was found distinct cavity formation in the epidermis, thus representing a real status spongiosus (epidermitis spongioculosa); Fig. 2 shows such a case. Such changes were not in any case detected in the control material (see later).

Case 12 a shows that the reaction as early as 7 hours after application of the patch is of almost purely lympho-histiocytic composition. It is, therefore, justifiable to believe that the reactions do not at any moment present a greater admixture of polymorphonuclear leukocytes.

Case 12 b shows that a vesicular reaction to chrome, in this case at any rate, is not eosinophilic, and that there is

an acute allergic inflammation of chiefly lympho-histiocytic structure.

Thus the investigations seem to show that an epicutaneous allergic skin reaction, whether it be of 7, 24, or 48 hours' standing, is attended by an inflammation in the skin, which according to its manner of origin must be termed acute but

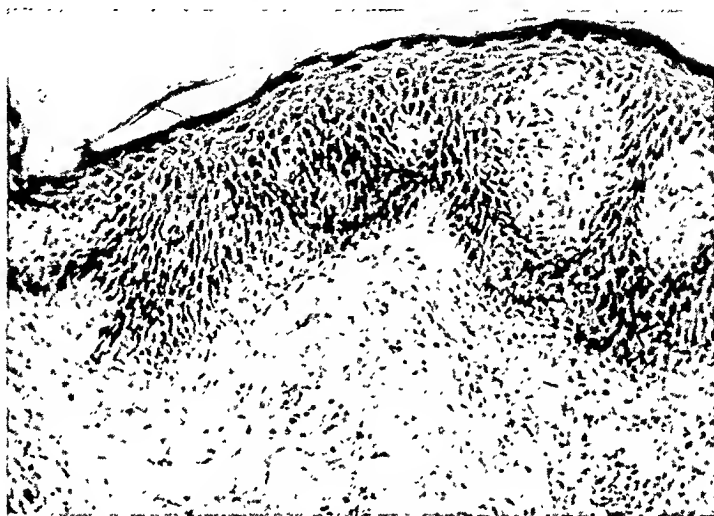


Fig. 2.

Case Nr. 12 b. Hematoxylin-eosin staining, enlarged about 190 times. Patch test with chrome 1 per mille. Biopsy after 7 hours. Status spongiosis in epidermis.

which differs from the majority of non-allergic acute inflammations by consisting chiefly of lympho-histiocytic cells; this applies both to the epidermal spongiosis and to the inflammatory infiltrations in corium and subcutis. The few intracutaneous allergic skin tests (Dmelcos test, trichophytin test, and Mantoux' test) elicit the same reaction as the epicutaneous tests.

These observations seem in a high degree to support *Bruun's* conception of the tissue reactions in acute allergic inflammations: The primary, purely allergic tissue processes consist — besides of stasis, edema, tissue swelling, and fibrinoid exudation — of a cellular infiltration which is chiefly

composed of lymphocytes and histiocytic cells. If a greater number of polymorphonuclear leukocytes is found, their existence must be considered as a secondary phenomenon due, for example, to necrosis or bacteria.

If one of the factors (for example necrosis), which are responsible for the appearance of polymorphonuclear leukocytes in the allergic cellular infiltrations, appears during the nascency of the allergic tissue reactions, primary and secondary allergic tissue processes will be seen at the same time, nay the secondary processes (the polymorphonuclear leukocytes) will even dominate the microscopic picture, as they do in the classical phenomenon of Arthus. However, in attenuated forms of Arthus's phenomenon, for example in allergic cutaneous reactions, provided that the antigens are applied appropriately, forms of inflammation will be seen in which the picture is dominated by the more purely allergic tissue processes. Both macro- and microscopically these reactions seem to be identical with the conditions known from allergic morbid pictures in Man.

It might be asserted that the lymphocytic inflammation infiltrates, which were found in these investigations, could be ascribed to the anestheticum applied (novocain-adrenalin), and thus be an effect of adrenalin. For, *Tonietti*¹⁹⁾ reports that the injection of adrenalin gives rise to lymphocytosis in the blood on account of sympathicus irritation. It might therefore be assumed that tissue lymphocytosis would appear, too. That is not the case, however. In the first place, many of the biopsies were carried out under conduction anesthesia far from the place of incision, and in the second place, the control experiments, in which biopsies were likewise performed under novocain-adrenalin anesthesia, chiefly presented cell reaction of polymorphonuclear leukocytes.

As a control experiment it was endeavoured in different ways to elicit an acute non-allergic and non-bacterial inflammation in skin and subcutis of 4 non-allergic persons (Nrs. 21—24). In 2 persons the inflammatory reaction was elicited by superficial freezing with carbon dioxide snow, in 1 person

Scheme II.

Pat. Nr.	Age (years)	Clinical diagnosis	Kind and standing of reaction	Differential count (5 fields altogether)	Microscopy
21	30	Nil	Freezing with earbon dioxide snow on right femur for 8 seconds. Biopsy after 24 hours	Among 240 cells were found 187 polymorphonuclear leukocytes and 53 lympho-histiocytic cells	There are a good many white blood corpuscles round vessels and lymph tracts. There are both lymphocytes and polymorphonuclear leukocytes. The latter preponderate, in isolated places even so much that there are only isolated lymphocytes in an infiltrate. <i>Eskelund.</i>
22	43	Nil	Freezing with earbon dioxide snow on right femur for 5 seconds. Biopsy after 24 hours	Among 340 cells were found 304 polymorphonuclear leukocytes and 36 lympho-histiocytic cells	Particularly in the uppermost part of eorium there is some leukocyte infiltration with considerable preponderance of the polymorphonuclear leukocytes; some lymphocytes are seen too. <i>Tellum.</i>
23	23	Non-allergic prurigo	24 hours' patch test with subliminate 2.5 per cent	Among 302 cells were found 202 polymorphonuclear leukocytes and 100 lympho-histiocytic cells	There seem to be necrotic echanges of the superficial layers. Along lymph tracts, round hair follicles, sebaceous and sweat glands, cellular infiltration with lymphocytes as well as polymorphonuclear leukocytes is seen. The latter seem to preponderate. <i>Eskelund.</i>
23	73	Psoriasis	Diathermy burn. Biopsy after 24 hours	Among 370 cells were found 288 polymorphonuclear leukocytes and 82 lympho-histiocytic cells	In an isolated place the surface epithelium is strongly necrotic, covered with fibrin; in the fibrin great quantities of polymorphonuclear leukocytes and some lymphocytes are seen. In the subjacent tissue is found scanty infiltration round the lymph vessels. Here, too, the infiltration consists essentially of polymorphonuclear leukocytes.

as a slight diathermy burn with a very fine electrode, and in 1 person as a corrosion with 2.5 % sublimate as patch test. Biopsy was carried out after 24 hours. The results are recorded in Scheme II.

Scheme II shows that, in 4 cases of acute non-allergic, non-bacterial inflammation, a form of reaction was found quite different from the above described acute allergic inflammation. In all the 4 cases the non-allergic infiltration was chiefly composed of polymorphonuclear leukocytes, even though there were many lymphocytes, too. The preponderance of the polymorphonuclear leukocytes in these experiments never was so conspicuous as the preponderance of the lympho-histiocytic cells in the allergy experiments, but still there is no doubt that there is a question of two histologically far different forms of inflammation, as is distinctly seen on comparison with Figs. 3 and 4. In none of the 4 control cases was status spongiosus seen in the epidermis or eosinophilic cells in the infiltrates.

One thing still remains unclear, namely, the importance of the eosinophilic leukocytes in allergic tissue reactions. It is a matter of fact — nor did I with the present work by any means intend to refute this fact — that in the phenomenon of Arthus an astonishingly great number of eosinophils may be found among the polymorphonuclear leukocytes. As was mentioned before, *Berger & Lang* by biopsy of an intracutaneous reaction in a flour allergic found up to 50 per cent of eosinophilic cells. Nor are eosinophilic cell infiltrates of rare occurrence in the lungs of asthmatics, and blood eosinophilia in allergics is found occasionally. However, eosinophilia is by no means a specific sign of allergy, since it may be met with in conditions which seem to have no connection with allergy at all. Moreover, *Miescher's* interesting report shows that patch tests with some substances, for example panthesin liniment, produce distinct tissue eosinophilia, whereas patch tests with other substances, for example salvarsan, do not entail eosinophilia, although there could be

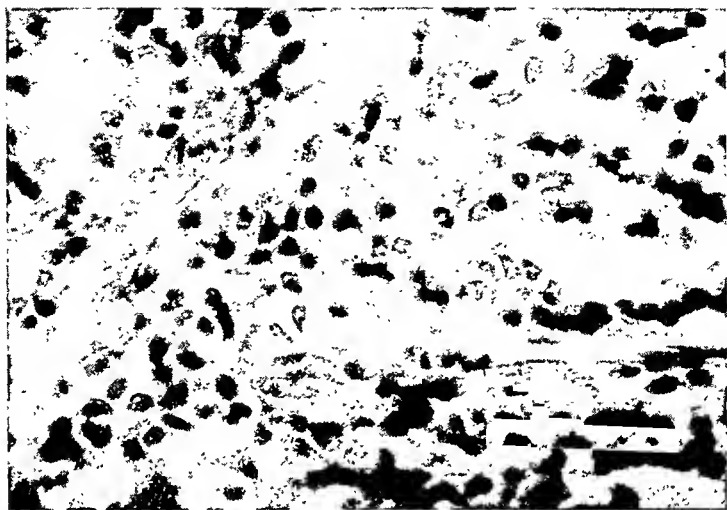


Fig. 3.

Case Nr. 3. Hematoxylin-eosin staining, enlarged about 945 times. Patch test with turpentine. Biopsy after 24 hours. Part of cell infiltrate in corium. Reaction of purely lymphohistiocytic cells.

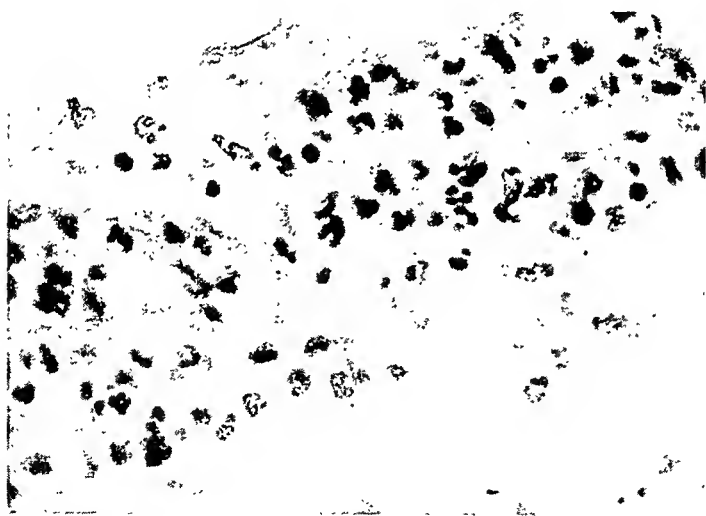


Fig. 4.

Case Nr. 22. Hematoxylin-eosin staining, enlarged about 945 times. Freezing of portion of femoral skin with carbon dioxide snow for 5 seconds. Portion from subcutis. Mixed cellular infiltration chiefly composed of polymorphonuclear leukocytes.

no doubt of the allergic nature of the positive salvarsan patch test. In a single one of the cases examined in the present work (Nr. 10, patch test for turpentine) only a few eosinophilic cells were found. On the whole, however, few or no eosinophilic cells were observed, and virtually no myeloid cells were found extravascularly. Consequently it is probable that the eosinophilic leukocytes must likewise be considered as a secondary allergic phenomenon.

Summary.

In 20 patients admitted to the dermatological department of the Rigshospital positive allergic skin reactions were found. Sixteen of the patients were submitted to epicutaneous patch tests, and the remaining four to intracutaneous tests.

Biopsy performed 24 hours after application revealed in all the cases an inflammatory reaction of chiefly lympho-histiocytic composition. In isolated cases biopsy was made 7 and 48 hours, respectively, after application. In these cases the cell infiltrates likewise consisted chiefly of lymphocytes and histiocytic cells. Few or no eosinophilic cells were observed.

There was no detectable difference between the cell infiltrates in the epicutaneously and the intracutaneously elicited reactions.

On the base of the present and of previous works the author thinks that the primary, purely allergic tissue reactions — besides of stasis, edema, tissue swelling, and fibrinoid exudation — consist of a cellular infiltration of almost purely lympho-histiocytic structure. Only if the primary allergic reactions are so intense as to give rise to necrotic processes, the polymorphonuclear leukocytes will appear in the place of inflammation, and then they will be able — as in the classical phenomenon of Arthus — to dominate the histological picture.

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